SHOUT
SHARE
IT

1143-103

THE BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

E. G. CONKLIN, Princeton University
E. N. HARVEY, Princeton University
SELIG HECHT, Columbia University
LEIGH HOADLEY, Harvard University
L. IRVING, Swarthmore College
M. H. JACOBS, University of Pennsylvania
H. S. JENNINGS, Johns Hopkins University
FRANK R. LILLIE, University of Chicago
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
T. H. MORGAN, California Institute of Technology
G. H. PARKER, Harvard University
A. C. REDFIELD, Harvard University
F. SCHRADER, Columbia University
DOUGLAS WHITAKER, Stanford University

H. B. STEINBACH, Washington University
Managing Editor

VOLUME 89
AUGUST TO DECEMBER, 1945

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

THE BIOLOGICAL BULLETIN is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to The Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $1.75. Subscription per volume (three issues), $4.50.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Massachusetts, between July 1 and October 1, and to the Department of Zoology, Washington University, St. Louis, Missouri, during the remainder of the year.

Entered as second-class matter May 17, 1930, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

LANCASTER PRESS, INC., LANCASTER, PA.

CONTENTS

No. 1. August, 1945

PAGE
Annual Report of the Marine Biological Laboratory 1
Whiting, Anna
  Dominant lethality and correlated chromosome effects in Habrobracon eggs x-rayed in diplotene and in late metaphase I 61
Harvey, Ethel Browne
  Stratification and breaking of the Arbacia punctulata egg when centrifuged in single salt solutions 72
Pace, D. M.
  The effect of cyanide on respiration in Paramecium caudatum and Paramecium aurelia 76
Metz, Charles B.
  The agglutination of starfish sperm by fertilizin 84
Kozloff, Eugene N.
  Cochliophilus depressus gen. nov., sp. nov. and Cochliophilus minor sp. nov., holotrichous ciliates from the mantle cavity of Phytia setifer (Cooper) 95
Scheer, Bradley T.
  The development of marine fouling communities 103
Spiegelman, S. and Florence Moog
  A comparison of the effects of cyanide and azide on the development of frogs' eggs 122

No. 2. October, 1945

Kidder, George W. and Virginia C. Dewey
  Studies on the biochemistry of Tetrahymena. IV. Amino acids and their relation to the biosynthesis of thiamine 131
LeFevre, Paul G.
  Certain chemical factors influencing artificial activation of Nereis eggs. 144
Hibbard, Hope and George I. Lavin
  A study of the Golgi apparatus in chicken gizzard epithelium by means of the quartz microscope 157
Hayashi, Teru
  Dilution medium and survival of the spermatozoa of Arbacia punctulata. I. Effect of the medium on fertilizing power 162
Kozloff, Eugene N.
  Heterocineta phoronopsidis sp. nov., a ciliate from the tentacles of Phoronopsis viridis Hilton 180
Abstracts of Scientific Papers Presented at the Marine Biological Laboratory, Summer of 1945 184

59265

H. S. Jennings, University of California
C. E. McClung, University of Pennsylvania
S. O. Mast, Johns Hopkins University
A. P. Mathews, University of Cincinnati
T. H. Morgan, California Institute of Technology
W. J. V. Osterhout, Rockefeller Institute
G. H. Parker, Harvard University
W. B. Scott, Princeton University

TO SERVE UNTIL 1948

Eric G. Ball, Harvard University Medical School
R. Chambers, Washington Square College, New York University
Eugene F. DuBois, Cornell University Medical College
Columbus Iselin, Woods Hole Oceanographic Institution
C. W. Metz, University of Pennsylvania
H. H. Plough, Amherst College
E. W. Sinnott, Yale University
W. R. Taylor, University of Michigan

TO SERVE UNTIL 1947

W. C. Allee, The University of Chicago
G. H. A. Clowes, Lilly Research Laboratory
P. S. Galtsoff, U. S. Fish and Wild Life Service
L. V. Heilbrunn, University of Pennsylvania
Laurence Irving, Swarthmore College
J. H. Northrop, Rockefeller Institute
A. H. Sturtevant, California Institute of Technology
Lorande L. Woodruff, Yale University

TO SERVE UNTIL 1946

Dugald E. S. Brown, New York University
E. R. Clark, University of Pennsylvania
Otto C. Glaser, Amherst College
E. N. Harvey, Princeton University
M. H. Jacobs, University of Pennsylvania
F. P. Knowlton, Syracuse University
Franz Schrader, Columbia University
B. H. Willier, Johns Hopkins University

TO SERVE UNTIL 1945

W. R. Amberson, University of Maryland School of Medicine
L. G. Barth, Columbia University
S. C. Brooks, University of California
W. C. Curtis, University of Missouri
H. B. Goodrich, Wesleyan University
R. S. Lillie, The University of Chicago
A. C. Redfield, Harvard University
C. C. Speidel, University of Virginia

EXECUTIVE COMMITTEE OF THE BOARD OF TRUSTEES

Lawrason Riggs, *Ex officio, Chairman*
E. N. Harvey, *Ex officio*
D. M. Brodie, *Ex officio*

Charles Packard, *Ex officio*
E. G. Ball, to serve until 1946
L. G. Barth
Robert Chambers, to serve until 1945
Wm. Randolph Taylor

The Library Committee

A. C. Redfield, *Chairman*
E. G. Ball
S. C. Brooks
M. E. Krahl
J. W. Mavor

The Apparatus Committee

D. E. S. Brown, *Chairman*
C. L. Claff
G. Failla
S. E. Hill
A. K. Parpart

The Supply Department Committee

D. A. Marsland, *Chairman*
P. B. Armstrong
P. S. Galtsoff
R. T. Kempton
Charles Packard

The Evening Lecture Committee

B. H. Willier, *Chairman*
M. H. Jacobs
Charles Packard

The Instruction Committee

H. B. Goodrich, *Chairman*
W. C. Allee
S. C. Brooks
Viktor Hamburger
Charles Packard, *Ex officio*

MARINE BIOLOGICA
WOODS HO
LIBRAR

The Buildings and Grounds Committee

E. G. Ball, *Chairman*
D. P. Costello
Mrs. E. N. Harvey
Roberts Rugh
Mrs. C. C. Speidel

II. ACT OF INCORPORATION

No. 3170

Commonwealth of Massachusetts

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips, and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, Henry B. Pierce, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Massachusetts, at 11:30 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Mass., at 10 A.M. Special meetings of the Trustees shall be called by the President, or by any seven Trustees, to be held at such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years; and in addition there shall be two groups of Trustees as follows:

(B) Trustees ex officio, who shall be the President and Vice President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer, and the Clerk;

(C) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next Annual Meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee *Emeritus* for life. The Trustees *ex officio* and *Emeritus* shall have all the rights of the Trustees except that Trustees *Emeritus* shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

IX. The Trustees shall have the control and management of the affairs of the Corporation; they shall elect a President of the Corporation who shall also be Chairman of the Board of Trustees; and shall also elect a Vice President of the Corporation who shall also be the Vice Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

X. Any person interested in the Laboratory may be elected by the Trustees to a group to be known as Associates of the Marine Biological Laboratory.

XI. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

XII. The account of the Treasurer shall be audited annually by a certified public accountant.

XIII. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen:

Herewith is my report as Treasurer of the Marine Biological Laboratory for the year 1944.

The accounts have been audited by Messrs. Seamans, Stetson, and Tuttle, certified public accountants. A copy of their report is on file at the Laboratory and inspection of it by members of the Corporation will be welcomed.

The principal summaries of their report—The Balance Sheet, Statement of Income and Expense, and Current Surplus Account—are appended hereto as Exhibits A, B and C.

The following are some general statements and observations based on the detailed reports:

I. Assets

1. Endowment Assets

As of December 31, 1944, the total book value of all the Endowment Assets, including the Scholarship Funds, was $983,900.57, a loss for the year of $19,808.06.

The Scholarship Funds were increased by the gift of $5,000.00 from Bishop James E. Cassidy of Fall River to establish the "Reverend Arsenious Boyer Burse." The principal losses incurred were due, as in the previous year, to the foreclosure of mortgage participations on New York City realty and the subsequent sale of the properties. In 1944 a four story tenement at 4856 Broadway on which the Laboratory held a mortgage investment of $30,057.10 was sold, the Laboratory receiving $6,026.00 cash and a new mortgage participation for $17,000, and sustaining a loss of $7,031.10. The property at 47 Murray Street, a five story loft building, was sold entirely for cash, at a loss of $9,569.24 on an investment of $21,928.75.

At the end of the year $803,403.76 was invested in marketable securities (bonds, preferred stocks and common stocks) with a market value of $825,005.80. $163,769.79 was invested in mortgage participations on New York City real estate and in real estate participations resulting from mortgage foreclosures. $16,727.02 was in uninvested principal cash.

The Treasurer's estimate of the actual value of the $163,769.79 in mortgage and real estate participations held on December 31 is $87,750.00. With the market value of $825,005.80 on marketable securities and the $16,727.02 in cash this makes a total current valuation of $929,482.82 compared with total book value of $983,900.57 and original capital value of $1,116,924.25.

2. *Plant Assets*

The total of Plant Assets (excluding the Gansett and Devil's Lane tracts) was $1,333,726.48 after deduction of $656,341.78 accumulated Depreciation Reserve, a decrease for the year of $7,699.40. Depreciation charges for 1944 were $26,929.31. The Reserve Fund was increased to a total of $16,895.62 by $3,529.41 transferred from current income (representing $279.41 profit on sale of Gansett lots, the Crane Company dividends, and part of the dividends on the General Biological Supply House stocks) and $93.99 interest received from the temporary investment of $10,000 of the Reserve Fund in U.S.A. Treasury bonds.

3. *Current Assets*

Current Assets including cash, inventories, and investments not in the Endowment Funds at cost, amounted to $202,239.67, an increase of $8,127.98. Current Liabilities totalled $2,181.09. The special reserve fund for repairs and replacements, made up of a portion of the 1943 income from the United States Navy rentals, and the value of certain equipment received from the Navy in lieu of restoration and repairs upon termination of the Navy lease, was $15,998.62 at the end of the year. Current Surplus was $184,059.96, $4,442.14 under the total for 1943.

II. Income and Expenditures

Total Income was $164,240.13, an increase of $4,943.19 over the 1943 income. Total Expenses were higher, $160,013.13, including Depreciation Reserves of $26,929.31 and special hurricane damage repairs of $2,466.17, but there was an actual net surplus of $4,227.00 for the year.

This surplus compares favorably with the $19,323.67 surplus in 1943 which resulted largely from the $20,150.00 rental from the United States Navy combined with reduced expenditures, and the deficit of $17,211.93 for 1942. Some of the reductions in 1944 income were a decline of $3,600.91 in endowment income, a loss of $6,000 in net income from the Supply Department compared with 1943, and a reduction of $2,286.00 in the dividends from the General Biological Supply House. The principal gains were Mrs. W. Murray Crane's gift of Otis Elevator stock valued at $2,325.00, and an increase of over $4,000 in net income from Research.

The income and expense items, although more normal than in 1943, still do not reflect what may be regarded as regular operations. Expenditures for equipment and necessary improvements, for example, are still unavoidably under what they should be to maintain the Laboratory at full efficiency. Some reserves have been built up for a few of these expenditures, but the Laboratory needs a larger endowment income to take care of maintenance.

EXHIBIT A

Marine Biological Laboratory Balance Sheet, December 31, 1944

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee		$ 968,737.59	
Securities and Cash in Minor Funds		15,162.98	
			$ 983,900.57
Plant Assets:			
Land	$ 111,425.38		
Buildings	1,327,675.21		
Equipment	186,122.42		
Library	329,639.23		
	$1,954,862.24		
Less Reserve for Depreciation	656,341.78	$1,298,520.46	
Reserve Fund, Securities and Cash		16,895.62	
Book Fund, Securities and Cash		18,310.40	
			$1,333,726.48
Current Assets:			
Cash		$ 27,513.52	
Accounts Receivable		12,357.71	
Inventories:			
Supply Department	$ 43,964.75		
Biological Bulletin	19,498.15	63,462.90	
Investments:			
Devil's Lane Property	$ 46,260.84		
Gansett Property	1,900.42		
Stock in General Biological Supply House, Inc.	12,700.00		
Other Investment Stocks	20,095.00		
Retirement Fund	12,966.30	93,922.56	
Prepaid Insurance		4,184.40	
Items in Suspense		798.58	
			$ 202,239.67
Total Assets			$2,519,866.72

Liabilities

Endowment Funds:			
Endowment Funds	$ 967,113.46		
Reserve for Amortization of Bond Premiums	1,624.13		
		$ 968,737.59	
Minor Funds		15,162.98	
			$ 983,900.57
Plant Funds:			
Donations and Gifts		$1,172,564.04	
Other Investments in Plant from Gifts and Current Funds		161,162.44	
			$1,333,726.48
Current Liabilities and Surplus:			
Accounts Payable		$ 2,181.09	
Reserve for Repairs and Replacements		15,998.62	
Current Surplus (Exhibit C)		184,059.96	
			$ 202,239.67
Total Liabilities			$2,519,866.72

EXHIBIT B

MARINE BIOLOGICAL LABORATORY INCOME AND EXPENSE, YEAR ENDED DECEMBER 31, 1944

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund		$ 27,291.16		$ 27,291.16
Library Fund		5,718.91		5,718.91
Donations		2,325.00		2,325.00
Instruction	$ 8,485.47	5,570.00	$ 2,915.47	
Research	4,981.44	13,654.38		8,672.94
Evening Lectures	45.85		45.85	
Biological Bulletin and Membership Dues	4,102.15	7,952.96		3,850.81
Supply Department	37,307.27	45,588.92		8,281.65
Mess	20,225.08	17,878.72	2,346.36	
Dormitories	30,761.47	13,190.82	17,570.65	
(Interest and Depreciation charged to above 3 Departments)	25,076.43			25,076.43
Dividends, General Biological Supply House, Inc.		16,510.00		16,510.00
Dividends, Other Investment Stocks		785.00		785.00
Rents:				
Bar Neck Property	759.46	4,800.00		4,040.54
Janitor House	21.35	360.00		338.65
Danchakoff Cottages	278.44	643.33		364.89
Rooms in Laboratory, Special		420.00		420.00
Sale of Library Duplicates and Micro Film		194.90		194.90
Microscope and Apparatus Rental		1,168.24		1,168.24
Sundry Income		187.79		187.79

Maintenance of Plant:				
Buildings and Grounds	18,759.11		18,759.11	
Apparatus Department	3,765.15		3,765.15	
Chemical Department	1,681.50		1,681.50	
Library Expense	6,756.08		6,756.08	
Workmen's Compensation Insurance	440.09		440.09	
Truck Expense	327.35		327.35	
Bay Shore Property	93.41		93.41	
Great Cedar Swamp	20.25		20.25	
General Expenses:				
Administration Expense	15,275.38		15,275.38	
Endowment Fund Trustee and Safe-Keeping	1,015.28		1,015.28	
Bad Debts	592.50		592.50	
Special Repairs on account of 1944 Hurricane Damage	2,466.17		2,466.17	
Reserve for Depreciation	26,929.31		26,929.31	
	$160,013.13	$164,240.13	$100,999.91	$105,226.91
Excess of Income over Expense carried to Current Surplus	4,227.00		4,227.00	
	$164,240.13		$105,226.91	

EXHIBIT C

Marine Biological Laboratory, Current Surplus Account, Year ended December 31, 1944

Balance January 1, 1944				$188,502.10
Add:				
Excess of Income over Expense			$ 4,227.00	
Gain on Gansett Lots Sold			176.04	
Bad Debts Recovered			37.64	
Reserve for Depreciation charged to Plant Funds			26,929.31	31,369.99
				$219,872.09
Deduct:				
Payments from Current Funds during Year for Plant Assets:				
Buildings		$ 3,064.00		
Equipment		1,542.52		
Library		5,559.12		
		$10,165.64		
Less Received for Plant Assets Sold		172.00		
			$ 9,993.64	
Pensions Paid		$ 3,460.00		
Less:				
Retirement Fund Income	$223.07			
Retirement Fund Gain on Securities	351.86			
Retirement Fund, Recovery on account of 1943 loss	.51	575.44		
			$ 2,884.56	

Transfers to Reserve Fund:			
Portion of Dividends from General Biological Supply House, Inc.	$ 2,500.00		
Dividends from Crane Company	750.00		
Profit on Gansett Lots for 1943	279.41		
		$ 3,529.41	
Building Fixtures and Equipment Received from First Naval District, transferred to Plant Funds.	$ 7,225.00		
Less Loss on Fixtures and Equipment Discarded	620.48		
		$ 6,604.52	
Repairs and Replacements Made by First Naval District during their occupancy of properties, set up as a Reserve		12,800.00	
			35,812.13
Balance, December 31, 1944			$184,059.96

Respectfully submitted,
DONALD M. BRODIE,
Treasurer

V. REPORT OF THE LIBRARIAN

The sum of $11,239.77 appropriated to the library in 1944 was expended as follows: books, $760.49; serials, $2,626.99; binding, $884.00; express, $60.14; supplies, $416.17; salaries, $6,239.77; back sets, $214.50; insurance, $50.00; sundries, $2.21; total, $11,254.27. The cash earnings of the library reverting to the laboratory were $194.90: from sale of duplicates, $38.73; microfilms, $144.86; serials lists, $11.31.

Of the Carnegie Corporation of New York Fund, $2,433.69 was expended for the completion of five and partial completion of nine back sets and two books.

The sum appropriated by the Woods Hole Oceanographic Institution for 1944 was $1,900.00. A balance of $263.08 remaining from 1943 made an available total of $2,163.08. Of this sum $113.69 was expended on current books and journals and $1,100.00 on salaries, leaving a balance of $949.39. A comparison of the amount spent on current books, journals and back sets during the pre-war years with that of the war years will show that this accumulating budget balance will be expended when the material for which it was designated shall have become available.

During 1944 the library received 678 current journals: 248 (10 new) by subscription to the Marine Biological Laboratory; 15 (none new) to the Woods Hole Oceanographic Institution; exchanges 201 (three new) with the "Biological Bulletin" and 23 (one new) with the Woods Hole Oceanographic Institution publications; 186 as gifts to the former and five to the latter. The Marine Biological Laboratory acquired 169 books: 119 by purchase of the Marine Biological Laboratory; six by purchase of the Woods Hole Oceanographic Institution; nine gifts from the authors, 22 from the publishers and 13 from miscellaneous donors. There were 18 back sets of serial publications completed: ten purchased by the Marine Biological Laboratory (five with the "Carnegie Fund"); two secured by exchange with the "Biological Bulletin"; one by exchange with the Woods Hole Oceanographic Institution publications; and five by duplicate material exchange and by gift. Partially completed sets were 59: purchased by the Marine Biological Laboratory, 23 (nine with "Carnegie Fund"); by exchange with the "Biological Bulletin," one; and by exchange of duplicate material and by gift, 35. In addition, 15 of the odd journal numbers presented by Dr. Dorothy R. Stewart (126 in all) were fitted into gaps in our sets.

The reprint additions to the library number 2,404: current of 1943, 401; current of 1944, 58; and of previous dates, 1,945. A total of 3,957 reprints, 1,321 not duplicates of our holdings, were presented to the library: 1,378 by Mrs. G. N. Calkins; 2,306 by Dr. Dorothy R. Stewart; 192 by Dr. Libbie H. Hyman; and 81 by Dr. D. A. Fraser.

It is with great pleasure that two very valuable gifts are acknowledged as presented to the library this year. Dr. Walter E. Garrey has presented his collection of reprints to be incorporated in the library's reprint holdings. As yet no count of these has been made. More detailed acknowledgment will occur in a later report. The same delayed account will be given of the reprints from Dr. E. B. Meigs' library, a gift of Mrs. Meigs. In addition to the reprints, Mrs. Meigs included in her gift long runs of fourteen different journals. As a further gift from Mrs. Meigs three of these sets will be bound and, with an appropriate book plate inserted, will be substituted for the old volumes now in the library.

At the end of the year 1944 the library contained 52,885 bound volumes and 133,054 reprints.

VI. THE REPORT OF THE DIRECTOR

To the Trustees of The Marine Bioogical Laboratory:

Gentlemen:

I beg to submit the following report of the fifty-seventh session of the Marine Biological Laboratory for the year 1944.

During the year the Laboratory has coped with difficulties brought on by the war—shortage of labor, and materials much needed for research and for the maintenance of the plant and the Supply Department—and with a hurricane which fortunately did not seriously damage our buildings. The immediate problem now reflects the encouraging change in the general situation throughout the country; it is to find laboratory space for the large number of investigators who expect to return in 1945.

1. *Attendance*

The anticipated increase in the number of investigators and students indicates that we have already passed the low point in the curve of attendance. This can be seen in the chart on page 20 which forms a part of the report prepared for the Committee of Review. Our numbers in the five year period from 1936–1940 were the greatest in the history of the Laboratory; in 1942 they decreased by nearly 50 per cent. The year 1943 showed a still further decline. A definite improvement is seen in the record of 1944, as shown in the Tabular View of Attendance on page 21. The prospects for a still larger number in 1945 are excellent.

From the curves on the chart, one can see the effect on attendance of changing conditions in the Laboratory and in the country at large. When new buildings were erected here, the attendance increased sharply. The first World War reversed the upward trend, but only for one year. The business depression, felt from 1932–1935, affected chiefly the number of "New Investigators." The present war has reduced our attendance to the level of 25 years ago. After 1945 there should be a rapid rise, but with our present buildings and equipment we cannot accommodate more investigators and students than we had in 1940.

New Investigators are those who come here for the first time; after the first year they are classified among "Returning Investigators." They are chiefly "Investigators under Instruction," that is, graduate students and Fellows. Over a long period of years they have constituted nearly one third of all investigators in attendance. Recently this proportion, as shown in the Tabular View of Attendance, has grown smaller; but actually, until the war, the number of graduate students, Fellows, and young instructors present each summer did not diminish. What happened was that many came as Research Assistants, probably for economic reasons.

Since 1941 the number of beginning investigators has declined by about 75 per cent from its previous level. The loss of so large a proportion of this important source of new members and future supporters of the Laboratory will be felt for many years. We fervently hope that those who are now prevented from working here will eventually return to us.

It is a pleasure to report that the number of institutions represented during the war years has not greatly diminished, and that the list of supporting institutions receives new additions every season.

2. *Laboratory Activities*

During the summer all of the usual activities of the Laboratory were carried on. After a lapse of one year, the weekly seminars were resumed, nine being held. In addition to these, several small groups of investigators met to discuss topics in which all were especially interested. It was the general opinion that more meetings of this kind should be held. All of the courses of instruction were given, the total registration of students being 75, a moderate increase over the preceding year. Dr. John B. Buck, who served for two seasons with signal success as head of the Invertebrate Zoology course, resigned at the end of the summer. The Committee on Instruction accepted his resignation with regret, and selected Dr. Frank A. Brown, of Northwestern University, to succeed him as instructor in charge.

3. *Associates*

The Trustees, at the regular meeting this year, directed the Executive Committee to appoint a committee to consider the advisability of establishing a new kind of membership in the Corporation, to which those interested in the welfare of the Laboratory might be elected. Mrs. W. Murray Crane, Mr. Lawrence Saunders, Dr. J. P. Warbasse (all of whom were elected to membership in the Corporation at the August meeting), Dr. G. H. A. Clowes, and the Director, were asked to discuss the matter. This committee felt that there are many people without special training in Biology, who have a sincere interest in the Laboratory, and that such friends would appreciate a formal connection with it. Following the Committee's approval of the plan, a special meeting of the Trustees was held in New York on December 9, 1944 to amend the By-laws so that this new type of membership could be made possible. The amendment which was adopted reads as follows: "Any person interested in the Laboratory may be elected by the Trustees to a group known as 'Associates of the Marine Biological Laboratory.'" It is hoped that both summer and permanent residents of Woods Hole and the vicinity may become members, and that friends in other parts of the country may also join.

At this special meeting the opinion was voiced by several Trustees that a winter meeting should be held regularly in order that current Laboratory problems could be discussed.

4. *The Committee of Review*

When the Friendship Fund in 1924 contributed a large sum to the Laboratory for endowment purposes, the Trustee of the endowment was directed to call once every ten years upon a Committee of Review to make a study of the work of the Laboratory. This Committee, which consists of nine members, includes a representative of the National Academy of Sciences, of the National Research Council, of the American Association for the Advancement of Science, and one professor of Biology from each of the following universities: Chicago, Columbia, Harvard, Pennsylvania, Princeton, and Yale. Its function is to determine whether the Laboratory continues to perform valuable services in biological research. The complete text of the Deed of Trust, in which the duties of the Committee are set forth, is printed in the 26th Annual Report which appears in Vol. 47 of the "Biological Bulletin."

The Committee first met in 1934 and voted that the Laboratory was satisfactorily fulfilling the purpose for which the endowment was given. The second decennial Committee met this year. Its findings, and the statement of the President and Director of the Laboratory regarding our activities during the years 1934–1943 are appended to this report. The Committee, in addition to its formal vote of approval, pointed out that in order to maintain a high level of usefulness, the Laboratory should secure additional funds for endowment and for purposes which are specified in their report. These recommendations, coming from a group of biologists, the majority of whom were not connected with the administration of the Laboratory, should be given most careful consideration.

5. *The Hurricane*

The September hurricane did not seriously damage our buildings. No water came in, as happened in the 1938 storm, but roofs and windows suffered. Some of the slate from the Dormitory and Apartment House roofs was blown off, many pieces imbedding themselves in distant houses. Fortunately no one was struck by these flying missiles. The Cayadetta wharf was practically demolished, and the sea wall badly broken by the tremendous waves that tossed great stones on to the street. The wharf has been partially restored by the Oceanographic Institution which has used it for the past two years. Had the full fury of the storm struck at high tide we might well have sustained a loss, due to sea water, even greater than that which we suffered in 1938. The wind, whose velocity far exceeded that of the previous hurricane, levelled a great number of trees in the Gansett and Devil's Lane tracts, on Dr. Clowes' property, and in the Fay Woods.

Against the destructive power of winds we can do little, but it is possible to protect the Brick Building from high water. The matter of increased protection should be given consideration.

6. *Loss by Death*

This year the Corporation has lost by death Prof. William Trelease who was elected in 1888 at the first regular meeting of the Trustees after the incorporation of the Laboratory.

7. *Gift*

The Laboratory acknowledges with sincere appreciation the receipt of 100 shares of Otis Elevator stock valued at $2,325.00, a gift of Mrs. W. Murray Crane.

8. *Election of Trustee*

At the meeting of the Corporation held August 8, 1944, L. G. Barth, Associate Professor of Zoology at Columbia University, was elected to fill the vacancy caused by the resignation of Prof. I. F. Lewis.

9. There are appended as parts of this report:

1. Memorial to Dr. Caswell Grave.
2. The Report of the Committee of Review.
3. The Decennial Review—Submitted to the Committee of Review.
4. The Staff.
5. Investigators and Students.
6. Tabular View of Attendance, 1940–1944.
7. Subscribing and Co-operating Institutions.
8. Evening Lectures.
9. Shorter Scientific Papers.
10. Members of the Corporation.

Respectfully submitted,
Charles Packard,
Director

1. Memorial to Dr. Caswell Grave

By Prof. R. A. Budington

It is with the greatest reluctance, and with true sorrow, that today we must include among those permanently lost to the Corporation the name of Caswell Grave. Those who knew him, as most of us here did, will miss his genial personality, with his habit of industry, his steady, keen interest in everything biological, his strict integrity of character; and the Board of Trustees will be very conscious of the absence of his sincere interest in the ongoing of the Laboratory, its policies, and its scientific significance.

Grave was born a Hoosier, on a farm in Monrovia, Indiana, and was very nearly 74 years of age at the time of his death at his home in Winter Park, Florida, last January 8th. He graduated from Earlham College in 1895, with Phi Beta Kappa rank; his alma mater honored him with her Doctor of Laws degree in 1928. His graduate studies were done at Johns Hopkins University, which conferred the Ph.D. in 1899. Meanwhile, he had spent summers at the Fisheries Bureau in Woods Hole, and at the Johns Hopkins Laboratory in Jamaica. After two further years of study as Bruce Fellow, he was appointed to the Hopkins teaching staff, a relation he continued for 18 years, for 13 of which he held the rank of Associate Professor. In 1919 he was appointed to the headship of the Zoological Department at Washington University, St. Louis, where the new Rebstock Laboratory had just been built. Here he gathered about him a staff of men of outstanding competency, and put the department on a basis widely recognized for scholarship and general efficiency.

Other responsibilities carried by Grave were: Director of the U. S. Fisheries Laboratory, Beaufort, N. C., 1902–1906; Shellfish Commissioner of Maryland, 1906–1912. In World War I he was ranked a captain in the Chemical Warfare Service. He was an active member of the AAAS; the American Society of Naturalists; a member of Sigma Xi; by turn he was Secretary-Treasurer, Vice President, and President of the American Society of Zoologists. As for the Marine Biological Laboratory, he was an outstandingly successful director of the Invertebrate Course from 1912–1917; a Trustee for 20 years, 1920–1940; thereafter, Trustee Emeritus. Few, if any, have taken the welfare of the Laboratory more seriously to heart than did he.

Grave's research interest embraced three quite different fields: pelecypod mollusca as to structure, physiology, and life histories; echinoderms, with special reference to embryology, and intraphyla relationships; while in later years he attacked the problem of metamorphosis in the ascidians, with special reference to the chemical factors retarding or accelerating it.

It is not too much to say that Caswell Grave was a wise man; and in the truest sense, in all that the appellations should imply, he was a "gentleman and a scholar." We are glad to pause and offer him such honor as we may, today.

August 9, 1944

2. MINUTES OF THE COMMITTEE OF REVIEW OF THE MARINE BIOLOGICAL LABORATORY

The Committee of Review provided for in the Deed of Trust Covering Funds for Endowment, Friendship Fund, Inc., and Central Hanover Bank and Trust Company of New York, met at the Marine Biological Laboratory, Woods Hole, Massachusetts, on August 9, 1944, at 9:00 A.M.

Mr. Lawrason Riggs, President of the Corporation, read the Call of Meeting, and commented on the history of the origin of the Deed of Trust, and on the duties of the Committee.

Present:

Professor W. C. Allee—representing The University of Chicago
Professor G. A. Baitsell—representing Yale University

Professor A. F. Blakeslee—representing The American Association for the Advancement of Science
Professor A. B. Dawson—representing Harvard University
Professor W. K. Gregory—representing The National Academy of Science
Professor R. W. Griggs—representing The National Research Council
Professor E. N. Harvey—representing Princeton University
Professor M. H. Jacobs—representing The University of Pennsylvania
Professor Franz Schrader—representing Columbia University

Dr. Blakeslee was elected Chairman of the Committee and (by invitation) Dr. Charles Packard, Director of the Laboratory, Secretary.

Dr. Packard presented the Decennial Review containing a brief statement of the activities of the Laboratory for the years 1934–1943, and called attention to the nine exhibits.

The Committee examined the exhibits, and after full discussion, unanimously

VOTED That the Marine Biological Laboratory is performing valuable services in biological research.

It was the opinion of the Committee that it could perform a useful service to the Laboratory by making suggestions regarding its future development.

VOTED That the Committee understands and appreciates the high quality of the Board of Trustees of the Laboratory, but thinks it desirable that each class of Trustees should contain at least one biologist not closely associated with the work of the Laboratory.

Moved and seconded that a recommendation be formulated that some means be considered for effecting more frequent changes in the Board of Trustees.

The motion was lost.

VOTED That the Chairman appoint a sub-committee of three to report to the full Committee on the specific needs of the Laboratory.

The Chairman appointed Drs. Harvey, Dawson, and Packard.

VOTED That the Chairman appoint a sub-committee of three to draft a statement in support of the first motion, this to follow in general the form of the report of the 1934 Committee of Review.

The Chairman appointed Drs. Schrader, Jacobs, and Baitsell.

Afternoon Session.

VOTED To accept and adopt the following statement in support of the first motion.

The Marine Biological Laboratory is performing valuable services in biological research. Its record is especially commendable in view of the difficult conditions experienced during the past ten years. Despite the steady decrease in income from endowments, and the more recent handicaps involved in war conditions, the scientific activities of the Marine Biological Laboratory have been maintained at a high level.

With marked decrease in attendance due to wartime conditions, the standards of the courses of instruction have been maintained.

The Library, already recognized as one of the foremost in its field, has on a reduced budget been steadily improved.

Important research continues to be done. To compensate for a decrease in attendance there has been some utilization of the Laboratory facilities for war work.

As in the past, one of the important features of the Marine Biological Laboratory has been the close association of investigators working in different fields. Likewise, cooperation and association with the Woods Hole Oceanographic Institution, as well as with the local station of the U. S. Fish and Wild Life Service, has increased to a laudable extent.

VOTED To accept and adopt, as amended, the following statement of the subcommittee on Laboratory needs:

1. The committee notes that the income of the Marine Biological Laboratory has decreased while the needs have continually mounted. The budget has been balanced at the expense of upkeep and necessary improvements. Obviously the setting up of a sufficient reserve for future developments has been impossible. Additional income is urgently needed for the following specific purposes:
   a. Replacement of apparatus, boats, and other equipment now becoming obsolete.
   b. Repair and renovation of buildings.
   c. Payment of subscriptions to foreign journals now held in Europe.
   d. Probable adjustment of salaries to meet increased cost of living.
   e. Additional pensions.
   f. A naturalist to replace Mr. G. M. Gray, now retired.
   g. A fireproof building to replace the present wooden Laboratory buildings.
2. The Committee recognizes that the acquisition of funds for the above purposes and for additional endowment constitutes the most important problem confronting the Trustees of the Laboratory. In view of the anticipated increase in research activity after the war, these needs appear to be immediate and imperative.

The Committee directed the Secretary to inform the Trustees of the Laboratory of the above matters. The condensed report will be forwarded to the Bank as Trustee of the Endowment Funds; the full minutes will be published in the 1944 Annual Report of the Director.

The Committee adjourned at 4:45 P.M.

CHARLES PACKARD,
Secretary

August 9, 1944

3. TO THE COMMITTEE OF REVIEW

Gentlemen:

The first decennial review (1923–1933) included the period of rapid growth of the Laboratory. The Endowment Fund was set up; the chief building erected, more than doubling the space available for research; a special endowment for the

Library permitted a notable addition to its holdings of journals and books; a large amount of apparatus and other tools of research became available. As a result, the scientific activity of the Laboratory increased greatly. Toward the end of the period, the economic depression brought about a temporary slowing down of growth.

In the period now under review (1934–1943) growth was resumed. The Library overflowed the space allotted to it and spread into the new wing, a gift of the Rockefeller Foundation. The number of investigators increased, exceeding all previous records. The war has temporarily ended this growth. The Library now receives few foreign journals; the younger investigators are in active service or in war research; the classes, which have been maintained without interruption, are almost devoid of men. But the Laboratory has continued to offer all of its usual facilities to investigators and students. The current year (1944) shows a marked upward trend in attendance and scientific activity. The stability of the Laboratory during these periods of war and economic depression is noteworthy.

Personnel

Many important changes in personnel have occurred in the past ten years. Dr. F. R. Lillie retired as President of the Corporation, and was elected President Emeritus. In his stead, Mr. Lawrason Riggs, the Treasurer since 1924, was chosen President; and the office of Vice President, created in 1942, was filled by Dr. E. N. Harvey.

The following changes have occurred in the Board of Trustees:

(a) Died in Office: C. R. Stockard, D. H. Tennent.
(b) Elected Trustees Emeritus (having reached the age of seventy years):

G. N. Calkins, d. 1943
E. G. Conklin
B. M. Duggar
W. E. Garrey
Caswell Grave, d. 1944
M. J. Greenman, d. 1938
R. G. Harrison
H. S. Jennings
C. E. McClung
S. O. Mast
A. P. Mathews
W. J. V. Osterhout
G. H. Parker
W. M. Wheeler, d. 1937

(c) Elected Trustees:

E. G. Ball, Assoc. Prof. Biol. Chem., Harvard Medical School
S. C. Brooks, Prof. of Zoology, University of California
D. E. S. Brown, Prof. of Physiology, N. Y. University Dental School
G. H. A. Clowes, Director of Research, Eli Lilly Laboratory
E. F. DuBois, Prof. of Physiology, Cornell Medical College
P. S. Galtsoff, Senior Biologist, U. S. Fish and Wild Life Service
Laurence Irving, Prof. of Biology, Swarthmore College
Columbus Iselin, Director, Woods Hole Oceanographic Institution
C. W. Metz, Prof. of Zoology, University of Pennsylvania
J. H. Northrup, Member, Rockefeller Institute

H. H. Plough, Prof. of Biology, Amherst College
Franz Schrader, Prof. of Zoology, Columbia University
E. W. Sinnott, Prof. of Botany, Yale University
A. H. Sturtevant, Prof. of Genetics, Calif. Institute Technology
W. R. Taylor, Prof. of Botany, University of Michigan
B. H. Willier, Prof. of Zoology, Johns Hopkins University

Dr. M. H. Jacobs, appointed Director in 1926, resigned in 1937. Dr. Charles Packard was made Assistant Director in that year, and Director in 1939. Since 1942 he has been Resident Director.

Our investigators and students are drawn from institutions widely distributed throughout the country (cf. map, Exhibit 3). In addition to universities and colleges, 36 Medical Schools and Hospitals have sent representatives; 9 Research Institutes, a number of Federal and State services, and industrial laboratories are also represented. A complete list of all of these various institutions is found in Exhibits 4 and 5.

Statistics of attendance for the period under review are shown in Exhibit 3. The chart indicates the annual attendance since 1888 when the Laboratory was founded. The term "New Investigators" refers to those who work here for the first time; "Returning Investigators" are those who have previously spent one or more seasons at the Laboratory. The effect of the first world war and of the present war; of periods of economic depression; and of expansion in research facilities, can be seen.

An incomplete list of publications from this Laboratory is found in Exhibit 8. The scientific record of students attending the courses for the years 1918–1931 is also a part of this Exhibit since it indicates their continuing interest and success in biological research and teaching.

The Laboratory is in full operation and is open for your inspection.

Respectfully submitted,

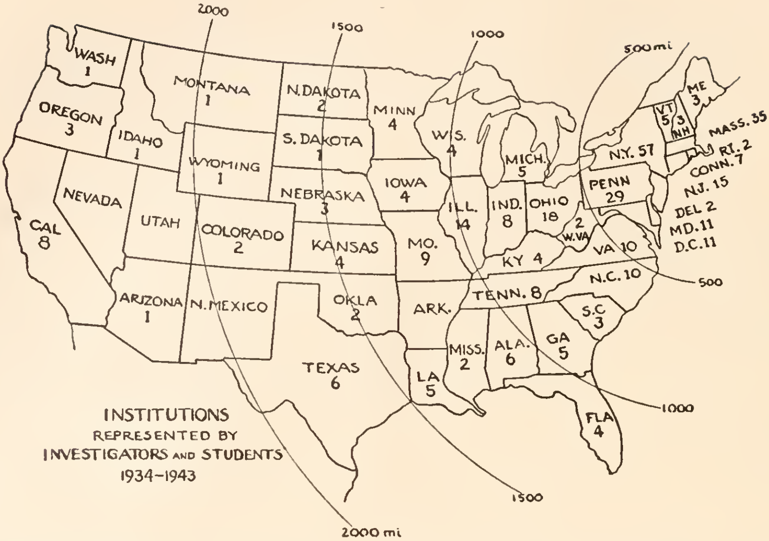
LAWRASON RIGGS, *President*
CHARLES PACKARD, *Director*

EXHIBITS

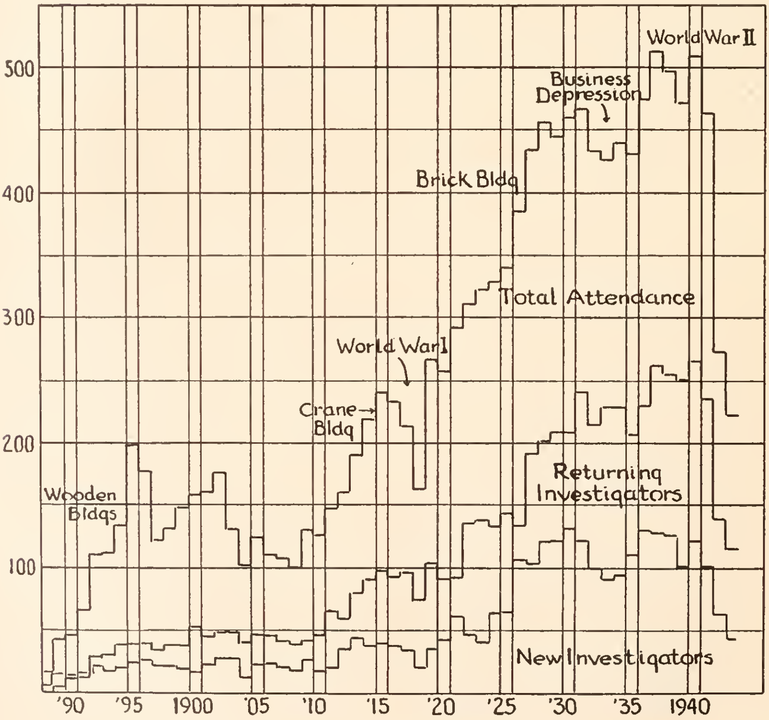
For the years 1934–1943 inclusive

1. Annual Reports
2. Annual Announcements
*3. Statistics of Attendance
*4. Institutions represented by Investigators and Students
*5. Subscribing and Cooperating Institutions
*6. Additions to the Library. Check List of Journals
7. Catalog of Investigators
8. Partial List of Publications from the Laboratory
9. The Scientific Record of Students attending the Courses

* These exhibits appear in this Report.


INSTITUTIONS
REPRESENTED BY
INVESTIGATORS AND STUDENTS
1934–1943
WASH 1
OREGON 3
CAL 8
IDAHO 1
NEVADA
MONTANA 1
WYOMING 1
UTAH
ARIZONA 1
N. MEXICO
COLORADO 2
N. DAKOTA 2
S. DAKOTA 1
NEBRASKA 3
KANSAS 4
OKLA 2
TEXAS 6
MINN 4
IOWA 4
MO. 9
ARK.
LA 5
WIS. 4
ILL. 14
MISS. 2
MICH. 5
IND. 8
OHIO 18
KY 4
TENN. 8
ALA. 6
GA 5
FLA 4
W.VA 2
VA 10
N.C. 10
S.C 3
PENN 29
N.Y. 57
VT 5
NH 3
ME 3
MASS. 35
R.I. 2
CONN. 7
N.J. 15
DEL 2
MD. 11
D.C. 11
500 mi
500
1000
1000
1500
1500
2000
2000 mi

Geographical distribution of institutions represented at the Marine Biological Laboratory 1934–1943



Attendance at the Marine Biological Laboratory 1888–1943

EXHIBIT 3

A TABULAR VIEW OF ATTENDANCE 1934–1943

	1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Investigators—Total	323	315	359	391	380	352	386	337	201	160
Independent	222	208	226	256	246	213	253	197	132	89
Beginning	49	56	76	74	53	60	62	59	16	19
Research Ass'ts	52	51	57	61	81	79	71	50	25	17
Library Readers								31	28	35
Students—Total	131	130	138	133	132	133	128	131	74	68
Botany	13	6	10	9	12	9	10	15	8	—
Embryology	30	33	34	35	34	36	34	37	24	13
Physiology	23	20	22	16	22	21	22	24	6	8
Protozoology	11	16	17	16	10	12	7	—	—	—
Zoology	54	55	55	57	54	55	55	55	36	47
Total Attendance less double registrations	439	429	473	511	496	471	507	461	273	222
Institutions represented	131	143	158	165	151	162	148	144	126	116
By investigators	98	111	120	134	125	132	112	102	83	71
By students	75	70	77	79	67	72	79	72	43	41
Schools and Academies										
By investigators	1	—	2	3	4	2	1	5	2	1
By students	5	3	3	2	1	2	2	2	—	1
Foreign Institutions										
By investigators	4	7	9	16	14	8	2	3	—	2
By students	1	1	5	—	3	1	1	1	—	—

EXHIBIT 4

Institutions Represented by Investigators and Students 1934–1943

A. Universities and Colleges

The figures after the name of the institution refer to the year the institution was first represented at the Laboratory

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Adelphi College	N. Y. '38					1					
Agnes Scott College	Ala. '15		1	1		1	1			1	
Akron, University of	Ohio '15	1									
Alabama, University of	Ala. '20		1	1	2			1	2		
Alabama University Medical	'37				1	1					
Alabama Polytech. Inst.	Ala. '38					1					
Albany Medical Coll.	N. Y. '31						1	1			
Albion College	Mich. '92		2	1	1		1				
American Internat. Coll.	Mass. '42									1	
American University	D. C. '31	3	2	2	1	1			1		
Amherst College	Mass. '13	2	5	11	10	6	8	6	6	7	1
Antioch College	Ohio '23				1						
Arizona, University of	Ariz. '25							1			
Assumption College	Mass. '42									1	
Atlanta University	Ga. '34	1	1	3	1		1			1	
Baldwin-Wallace Coll.	Ohio '35		1								
Bard College	N. Y. '35		1	1	2	1					
Barnard College	N. Y. '96	6	3	4	4	3	1	3	2		1
Baylor Univ. Medical	Tex. '42									1	
Beloit College	Wis. '96							1			
Bennington College	Vt. '35		2					1		1	1
Berea College	Ky. '28		1								
Birmingham-South. Univ.	Ala. '26						1				
Boston College	Mass. '38					1					
Boston University	Mass. '17						1				
Boston University Medical	Mass. '37				1						
Boston Teachers Coll.	Mass. '37				1						
Bowdoin College	Me. '93	2	1	1	1	1	1	1	1	1	
Bradley Poly. Inst.	Ill. '96	1									
Bridgewater State T. C.	Mass. '38					2					
Brooklyn College	N. Y. '32	7	5	9	7	2	4	7	3	1	1
Brown University	R. I. '90	1		1	3	3	7	7	7	1	

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Bryn Mawr College	Penn. '88	1	1		1		5	2	4		
Buffalo, University of	N. Y. '95						2				
Buffalo University Medical	N. Y. '39						1				
Butler University	Ind. '16		2	1							
California Inst. Tech.	Cal. '29	2	3	2	6	3	3	2	4	2	2
California, Univ. of	Cal. '00		1	3		2	3	5	4	5	1
Canisius College	N. Y. '37				1	1	2	4	2	4	
Carnegie Inst. Tech.	Penn. '09	1			1						
Catholic Univ. of Amer.	D. C. '42									2	2
Central State T. C.	Okla. '36			1							
Centre College	Ky. '37				1						
Charleston, Coll. of	S. C. '06			1			1	1			1
Chestnut Hill College	Penn. '41								2	2	
Chicago, University of	Ill. '92	10	7	6	7	9	5	13	8	2	3
Chicago University Medical	Ill. '40							1	1		
Cincinnati, Univ. of	Ohio '92	7	9	7	8	7	5	3	7	1	1
Cincinnati Univ. Medical	Ohio '35		3	2	3	3	5	3	1		
Clark University	Mass. '88	1	2	1	1	1	2				
Coe College	Iowa '20									1	
Colby College	Me. '99			1							
Colgate University	N. Y. '98					1					
College of Scholastica	Minn. '39						1				
College City of N. Y.	N. Y. '94	7	2	6	8	5	3	5	5		
Colorado, Univ. of	Col. '14	2	1								
Colorado Univ. Medical	Col. '25							1			
Columbia University	N. Y. '91	28	25	22	20	21	18	18	11	8	5
Columbia University Medical	N. Y. '94	5	5	5	7	8	5	3	2	5	4
Connecticut College	Conn. '20			2	2	2	3	3	2	2	1
Converse College	S. C. '38					1					
Cornell University	N. Y. '91	1		2	2	3	3	3	5	4	1
Cornell University Medical	N. Y. '09	12	12	9	11	8	10	6	5	1	1
Dartmouth College	N. H. '96	4	3	4	4	4	4	4	3	2	1
Davis and Elkins Coll.	W.Va.'41								1		
Delaware, Univ. of	Del. '98							1			
Delta State T. C.	La. '36			1							
DePaul University	Ill. '41								1		

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
DePauw University	Ind. '89	7	6	7	3	4	2	5	3	1	3
Dillard University	La. '42									1	1
Drew University	N. J. '38					1	2	1		2	
Drury College	Mo. '43										1
Duchesne College	Neb. '35		1								
Duke University	N. C. '25	5	2	1	8	7		3	2	1	1
Earlham College	Ind. '01				1		1				
East Carolina T. C.	N. C. '38					1					
Edgewood Park Jr. Coll.	N. C. '34	1									
Elizabethtown Coll.	'38					1					
Elmira College	N. Y. '21					1	2				
Elon College	N. C. '40							1			
Emory University	Ga. '97			1			2				
Emory Junior Coll.	Ga. '33				1						
Fisk University	Tenn. '31							1			
Flat River Jr. Coll.	'28			1							
Flora Macdonald Coll.	N. C. '25				1						
Florida, University of	Fla. '41								1		
Florida State College	Fla. '39						1				
Florida Coll. for Women	Fla. '29						1				
Fordham University	N. Y. '19								3	3	8
Franklin Marshall Coll.	Penn. '91			1			1	1	1		
George Washington Univ. Medical	D. C. '14	1	1	1	1	1	2	2	1		2
Georgia, University of, Medical	Ga. '37				1						
Gettysburg College	Penn. '39						1	1	2		
Goucher College	Md. '94	3	2	4	3	4	5	3	3	2	3
Great Falls Norm. Coll.	Mont. '38					1	1	1			
Grinnell College	Iowa '95	1									
Gulf Park College	Ky. '36			1							
Hahneman Medical Coll.	N. Y. '38					1	1	2			
Hamilton College	N. Y. '91		2	2	2		1		1		
Harvard University	Mass. '89	10	14	11	15	11	16	12	14	4	1
Harvard University Medical	Mass. '89	1	2	3	2	4	2	6	3	3	2
Haverford College	Penn. '89								1		
Heidelberg College	Ohio '91									1	
Herzl Junior College	Ill. '43										1

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Hood College	Md. '15	1									
Howard University	D. C. '09	1	1	1	2		1				
Hunter College	N. Y. '14	7	3	4	2	1	4	1		3	
Illinois, Univ. of	Ill. '90	5	6	2	4	2	1	5	4	3	2
Indiana University	Ind. '89			1				2	2		
Iowa, State Univ. of	Iowa '94	6	3	6	3	3	5	5	6	2	1
Iowa, State College of	Iowa '19	2	1	3	3	3	5	1	1		
Johns Hopkins Univ.	Md. '89	9	3	8	11	17	12	10	11	8	2
Johns Hopkins Univ. Medical	Md.	2	2	4	2	4	5	1			
Kansas, University of	Kan. '90	1		2	1			1			
Kansas State College	Kan. '26									1	
Kansas State T. C.	Kan. '34	1									
Kent State University	Ohio '30							1			
Kenyon College	Ohio '96				1	1					
Lander College	S. C. '38					1					
LaVerne College	Cal. '34	1									
Leland Stanford Univ.	Cal. '91			1		1	3	7	1	1	
Lincoln University	Penn. '01		1								
Long Island Univ.	N. Y. '29	3	3	3	2	1	1	1		1	
Long Island Univ. Medical	N. Y. '19									1	
Loyola Univ. Medical	La. '31	1		1					1		
Maine, University of	Me. '95	1	1	1	1						
Marquette University	Wis. '36			1							
Maryland, Univ. of	Md. '41								1		
Maryland Univ. Medical	Md. '96			8	11	13	11	4	3	5	1
Mass. State College	Mass. '89				1	1	1	1	2	2	1
Mass. Inst. Technology	Mass. '88	1					1	1	2	2	1
Miami University	Ohio '91				1	3	3	3	3	2	
Michigan, Univ. of	Mich. '88	4	3	2	2	4	3	6	9	2	2
Michigan Agric. Coll.	Mich. '10					1					
Middlebury College	Vt. '95		1			1	1				
Middlesex College	Mass. '37				1						
Minnesota, Univ. of	Minn. '98		1	2	7	3	2	3	2	1	
Mississippi, Univ. of	Miss. '99							1			
Missouri, Univ. of	Mo. '95	3	7	10	1	7	1	5	4	1	
Missouri State T. C.	Mo. '43										1

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Moberly Junior Coll.	Wis. '40							1			
Morehouse College	Ga. '27						1				
Morristown College	Tenn. '37				1						
Mt. Holyoke College	Mass. '88	3	3	6	7	8	7	4	8	8	3
Mt. Mercy College	Penn. '39						1				
Mt. St. Louis Coll.	'34	1									
Mundelein University	Ill. '39						1		1		
National Park Coll.	Md. '42									1	
Nebraska, Univ. of Medic.	Neb. '34	1									
New Hampshire State U.	N. H. '00					1					
New Jersey College for Women	N. J. '28	4	3	2	1	3	1		1		1
New Jersey State T. C.	N. J. '31	2	4	6	5	3	4	3	1		
New Rochelle, Coll. of	N. Y. '34	1				1	1				1
New York University	N. Y. '96	4	3	10	7	7	2	5	7	2	2
New York University Medical	N. Y. '25	6	2	6	10	7	3	9	5	7	6
New York University Wash. Sq.	N. Y. '24	10	10	10	10	11	10	12	8	9	5
Newark, University of	N. J. '41								1		
Newark State T. C.	N. J. '41								1		
North Carolina, Univ. of	N. C. '99		1	1	1	2	3	1	3		3
North Carolina Coll. for Negroes	N. C. '40							1			
N. C. State College	N. C. '31		1	2							
N. C. Womens College	N. C. '22			1	1						
N. Dakota State Univ.	N. D. '93	2	1	1							
N. Dakota Agric. Coll.	N. D. '23		1					1			
N. Texas Agric. Coll.	Tex. '38					1					
Northwestern Univ.	Ill. '93	3	4	3	6	1	5	9	2		
Notre Dame Univ.	Ind. '21			1	1	1	1				
Oberlin College	Ohio '90	5	7	8	7	5	4	6	6	4	5
Ohio State Univ.	Ohio '90			1	4	10	3	7	8	5	2
Ohio University	Ohio '14				2						
Ohio Wesleyan Univ.	Ohio '91				1	1	2		2		1
Oklahoma, Univ. of	Okla. '09						1	1			
Oklahoma City, Univ. of	Okla. '37				1	1	1	1	1		
Pennsylvania, Univ. of	Penn. '91	37	38	35	31	28	26	32	27	17	17
Pennsylvania, Univ. of Medical	Penn.	6	3	5	6	5	8	8	6	6	3
Penn. Coll. for Women	Penn. '01	3	3	1	1	2	2	2		2	1

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Penn. State Coll.	Penn. '07						1				
Pittsburgh, Univ. of	Penn. '21	3	5	7	7	4	5	7	6	2	
Placer Junior College	Cal. '40							1			
Pomona College	Cal. '24			1							
Princeton University	N. J. '90	12	7	12	13	12	13	9	9	3	5
Providence College	R. I. '35		1	2							
Purdue University	Ind. '28		3	5	4	5	1	3	3		
Queens College	N. Y. '28					1	1	4	3		
Radcliffe College	Mass. '95	4	3	3	6	2	1	2	5	2	1
Randolph-Macon Coll.	Va. '89								1		
Reed College	Ore. '39						1				
Rensselaer Poly. Inst.	N. Y. '36			1	1				1		
Rice Institute	Tex. '16				1						
Richmond, College of	Va. '13				1	1			1	1	1
Rochester, Univ. of	N. Y. '92	8	10	4	7	5	5	6	6	2	4
Rochester, Univ. of Medical	N. Y. '35		5		2	2	1				
Russell Sage College	N. Y. '20				1		2	2	1	5	4
Rutgers University	N. J. '14	3		2	1	1	2	1	2	2	
St. Francis Xavier Coll.	Ill. '14	1		2							
St. Johns College	Md. '34	2	2	1	2	2	1				
St. Louis University	Mo. '03	1	1	2	3						
St. Louis University, Maryville	Mo. '37				1						
St. Thomas College	Minn. '35		1								
St. Vincent College	Penn. '24								1		
Sarah Lawrence College	N. Y. '32		4	5	1				2	1	
Seton Hall College	N. J. '35		1	2							
Seton Hill College	Penn. '29				1	1		1	3	1	4
Simmons College	Mass. '07	1	1	1	1	1		1			
Skidmore College	N. Y. '22	1	2	2	2				1	1	
Smith College	Mass. '92	1	7	5	2	2	2	2	5	4	3
J. C. Smith University	N. C. '34	1	1	1	1	2					
Southern California, University of	Cal. '96						2				
Southern Oregon State Normal	Ore. '38					1					
Southwestern Univ.	Tenn. '22		1					1			
Spring Hill College	Ala. '38					1					
Springfield College	Mass. '40							2	1		
Stephens College	Mo. '36			1							

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Swarthmore College	Penn. '88	6	4	7	6	11	9	7	4		1
Sweet Briar College	Va. '09		2							1	
Syracuse University	N. Y. '94		1	1	2			1	1	1	
Syracuse University Medical	N. Y. '99	2	2	1	1	1	2	4	2	1	
Temple University	Penn. '97	2	2	1	4	3	2	3	4	2	3
Tennessee, Univ. of	Tenn. '95	3									
Tennessee, Univ. of Medical	Tenn. '35		2	2	2						
Texas, University of	Tex. '95					1		1	1		
Texas, University of Medical	Tex. '36			1	1	1					
Texas Christian Univ.	Tex. '21									1	
Toledo, Univ. of	Ohio '17					2	2		1		
Tougaloo College	Miss. '39						1				
Trinity College	Conn. '00	1	2	2	2	2	2	1	1	1	
Tufts College	Mass. '92			1			2	1	2		2
Tulane University	La. '16								1		
Tulane Newcomb Coll.	La. '13	1							1		
Union College	N. Y. '15	2	1	2	1	3	2	4	3	2	1
Union College	Ky. '39						1				
Ursinus College	Penn. '95		1								
Vanderbilt University	Tenn. '91		1					1			
Vanderbilt University Medical	Tenn. '34	4	3	3	3	4	3	2	1	2	1
Vassar College	N. Y. '88	4	2	3	2	6	3	5	7	6	6
Vermont, University of	Vt. '15		1					1			
Vermont, University of Medical	Vt. '38					2	1	1	1	1	1
Vermont State Normal	Vt. '40							1	1	1	1
Villanova College	Penn. '39						1	6	5	1	
Virginia, Univ. of	Va. '91		2	1	1	2	2				
Virginia, Univ. of Medical	Va.	2	2	2	4	2	2	2	1		
Virginia State T. C.	Va. '34	1									
Virginia Intermont Coll.	Va. '41								1		
Wabash College	Ind. '07	1	2	3	1	1		2	2		
Washburn College	Kan. 40'							1			
Washington University	Mo. '00	1	2	8	12	9	10	8	5	5	9
Washington University Medical	Mo.		2		6	3	2	3	1		
Washington, Univ. of	Wash. '15					1					
Washington and Jefferson College	Penn. '11	1	1	1	1	2	1	2	1	2	1

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Washington and Lee	Va. '17			1							
Wayne University	Mich. '34	1	1								
Wellesley College	Mass. '88	3	5	3	3	4	2	2	2	5	1
Wesleyan University	Conn. '89	6	5	3	5	6	4	7	10	3	1
West Virginia Univ.	W.Va. '01	1					1		1		
Westbrook Junior Coll.	Conn. '40							1			
Western College	Ohio '98					1					
Western Reserve Univ.	Ohio '95	3	1			4	1		1		
Wheaton College	Mass. '18	1	2	1	1	4	3	4	2	2	3
Whitman College	Ore. '43										2
William and Mary	Va. '22			1	1	1	1	1	1		
Williams College	Mass. '92	1	4	2	1	5	4	3	5	1	
Wilson College	Penn. '24	1	1	2	2	2	1	1	1	1	1
Wisconsin, Univ. of	Wis. '98	4	4	2	5		1	2	2		2
Womens Medical College	Penn. '92	1		3	3		2	3			
Wooster College	Ohio '13				1						
Wright Junior College	Ill. '41								1		
Wyoming, Univ. of	Wyo. '29								1		
Yale University	Conn. '91	14	8	6	16	10	9	11	15	5	1
Yale University Medical	Conn. '38					1		4	2	1	

B. HIGH SCHOOLS, ACADEMIES, ETC.

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Abraham Lincoln H. S.	N. Y.			1							
Agnes Irwin School	N. Y.			1							
Annapolis H. S.	Md.	1									
Berkshire School	Mass.								1		
Birmingham H. S.	Ala.	1									
Boston H. S.	Mass.	1							1		
Bronxville H. S.	N. Y.		1								
Caldwell H. S.	N. J.			1							
Central H. S.	D. C.				1						
Chicago H. S.	Ill.						1				
Choate School	Conn.							1	1		
Dana Hall	Mass.			1							
Darrow School	N. Y.						1				
Deerfield Academy	Mass.						1				

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Eastern District H. S.	N. Y.				1						
Emma Willard School	N. Y.										1
Exeter Academy	N. H.							1	1	1	1
Galesburg H. S.	Ill.	1									
Grand Falls H. S.	Can.				1						
Groton School	Conn.			1						1	
Hallahan H. S.	Penn.							1			
Hawthorne H. S.	N. J.			1							
Hyde School	Mass.			1							
Hyde Park School	Ill.				1			1	1		
Jenkintown H. S.	Penn.		1								
Knox School	N. Y.			1							
Lawrenceville School	Mass.								1		
Los Angeles H. S.	Cal.								1		
Milton Academy	Mass.	1		1		4	4	5	4		
Nativity H. S.	Mass.							1			
Negaunee H. S.	Mich.	1									
Oradell H. S.	N. J.					1					
Pennsgrove School	Penn.								1		
Potomac School	D. C.								1		
St. Andrews School	Del.						1				
St. Catherine School	Va.							1			
St. Joseph's Seminary	N. Y.									1	
St. Mary of the Lake Sem.	Ill.								1		
Scott, H. S.	Ohio	1									
Society of the Divine Word	Mass.									1	
Straubenmiller H. S.	N. Y.				1						
Theo. Roosevelt H. S.	N. Y.			1	1						
Union H. S.	N. J.	1									
Union City H. S.	Tenn.	1									
University H. S.	Minn.		1								
Vineland Training School	N. J.		1	1							
Walton H. S.	N. J.				1						
Washington H. S.	D. C.						1				
Weequahic H. S.	N. J.			1	1	1				1	
Westtown Friends School	Penn.							1			

EXHIBIT 4—*Continued*

C. Institutes, Foundations, etc.

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
American Mus. Nat. Hist.	N. Y. '09		1								
Arlington Chemical Co	'31	1	1								
Barnard Skin and Cancer Hospital	Mo.								1		
Beth Israel Hospital	N. Y. '42										
Biol. Institute, Phil.	Penn. '39						1				
Carnegie Institute											
Cold Spring	N. Y. '14		1								
Washington	D. C. '15	2				4	2	1	1		
Frick Education Comm.	'42									1	
Guggenheim Foundation	'40							1			
Guggenheim Dental Clinic	'43										1
Journ. Industrial and Engineering Chemistry	'28	1	1	1	1	1	1	1	1		
Internat. Cancer Research Foundation	'37				1						
Eli Lilly Company	Ind. '19	5	4	4	4	5	5	4	5	5	2
Marine Studios, Inc.	Fla. '42									1	
Memorial Hospital	N. Y. '26	2	1	1	2	3	3	2	1	1	1
Mt. Sinai Hospital	N. Y. '40							2	1	1	1
Nat. Cancer Institute	Md. '39						2				
Nat. Research Council	D. C.				1					1	
N. Y. State Agricult. Station	N. Y. '18				1						
N. Y. State Dept. Health	N. Y. '19	1	2	1	1	1	1	2	2	1	
Overly Biochemical Research Found.	N. Y. '43										1
Phila. Acad. Nat. Sci.	Penn. '89					1		1			
Rockefeller Institute	N. Y. '11	10	12	11	11	13	8	14	9	6	5
Rockefeller Foundation Fellowship		3		1		2	1				
Russell Sage Institute of Pathology	N. Y. '34	1					1	1			
St. Luke's Hospital	N. Y. '40							1			
U. S. Dept. Agriculture	D. C. '99					1					
U. S. Dept. Public Health	D. C. '20					1					
U. S. Fish and Wild Life Service	D. C. '42									2	2
Wistar Institute	Penn. '08	1	1	1	1		1				
Woods Hole Oceanographic Inst.	Mass. '43										

EXHIBIT 4—*Continued*

D. INSTITUTIONS OUTSIDE THE UNITED STATES

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Austria	Univ. of Innsbruck	1									
	Univ. of Vienna					2					
Belgium	Univ. of Ghent					1					
	Univ. of Liege					1					
	Belgian-American Educ. Foundation						3				
British Isles	Queens Coll. Belfast			1							
	Cambridge University	1			1	1	1				
	Trinity Coll. Dublin				1						
	Univ. of Edinburgh					1					
	Univ. of Leeds				1						
	Univ. of London				2			1	1	1	1
	Univ. Coll. London			1		1					
	Univ. Coll. Nottingham							1			
	Oxford University			1		1					
	British Fish. Service					1					
Canada	Acadia University N. S.	1									
	Univ. British Columbia			1							
	Dalhousie University, N. S.	2				1	1		1		
	McGill University, Ont.			1	2	3	2	3			1
	Univ. of Manitoba				1						
	Univ. of Montreal	1	1	1		1		1			
	Memorial University, N. F.		1								
	Coll. Ste. Marie, Montreal		1								
	Univ. of Toronto	6	7	6	3	5	4	5	4	4	1
	Univ. West. Ontario		1	1							
	Royal Soc. Canada			1							
Far East	Womens Medical College, Madras, India						1				
	Judson Coll. Rangoon, Burma						1				
China	Peking Union Med. Coll.						1				
Colombia	Cotton Res. Station										1
Denmark	Carlsberg Laboratory		1					1			
	Univ. of Copenhagen							1	1		
Egypt	Egyptian Educ. Commission		1								

EXHIBIT 4—*Continued*

		1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
France	Pasteur Institute, Paris					1					
	University of Paris				1						
	University of Strasbourg				1						
	University of Strasbourg Medical				1						
Germany	University of Berlin			1	1	1					
	Neurolog. Inst. Frankfurt				2						
	University of Munich			1							
Hungary	University of Debrescen				1						
	Budapest Univ. Medical		1								
	Franz Joseph University					1					
Italy	University of Padua		1		1						
Japan	Misaki Biolog. Inst.			1							
Norway	University of Oslo							1			
Peru	Guano Administration									1	
Poland	University of Lwow		1		1						
Russia	Moscow, Inst. Genetics			2							
Serbia	Belgrade Medical Coll.		1		1						
Spain	Barcelona Medical Coll.			1							
	University of Lund		1		1						
Sweden	Karolinska Inst. Stockholm		1		1						
	University of Stockholm	1		1							
Switzerland	Physiological Inst. Berne	1									
	Zoological Inst. Berne				1						
	University of Geneva			1							
Uruguay	Ministry of Pub. Health										1
Cuba	University of Havana					2	2				

Summary

	1923–33	1934–43
Universities and Colleges	246	269
High Schools and Academies	30	50
Institutes, Foundations, etc.	56	31
Foreign Institutions	101	59
	433	409

EXHIBIT 5

Subscribing and Cooperating Institutions

A cooperating institution is one that has subscribed for the two preceding years, or that announces its intention of subscribing regularly. A subscribing institution is one that pays for one or more tables or rooms.

	1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
American University	x		x	x	x			x		
Amherst College	x	x	x	x	x	x	x	x	x	x
Atlanta University	x	x	x	x		x			x	x
Barnard College	x	x			x		x	x		
Belgian-Amer. Educ. Found.					x	x				
Bell Telephone Laboratory										x
Berea College	x	x								
Beth Israel Hospital									x	
Biological Institute, Phila.							x	x	x	x
Bowdoin College	x	x	x	x	x	x	x	x	x	x
Brooklyn College							x	x	x	x
Brown University				x	x	x	x	x	x	
Bryn Mawr College	x	x	x	x	x	x	x	x	x	
Buffalo University Medical						x				
Butler University		x								
C. R. B. Educational Found.				x						
California Inst. Technol.		x	x	x	x	x	x	x		
Canisius College						x	x	x	x	
Carnegie Inst. Washington					x	x				
Catholic Univ. of America										x
Chicago, University of	x	x	x	x	x	x	x	x	x	x
Chicago, University of Medical		x								
Children's Hospital Cincinnati		x								
Chinese Educational Mission	x									
Christ Hospital, Cincinnati		x								
Cincinnati, University of	x	x	x	x	x	x	x	x	x	x
Columbia University	x	x	x	x	x	x	x	x	x	x
Columbia University Medical		x		x	x	x	x	x	x	x
Commonwealth Fund	x									x
Connecticut College					x	x				
Cornell University	x	x	x	x			x	x	x	x
Cornell University Medical	x	x	x	x	x	x	x	x	x	x
Dalhousie University	x				x	x				
Dartmouth College				x	x	x				
De Pauw University	x	x	x	x	x	x	x	x		
Drew University					x	x			x	

EXHIBIT 5—*Continued*

	1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Duke University	x	x	x	x	x	x	x	x	x	x
Eli Lilly Research Lab.	x	x	x	x	x	x	x	x	x	x
Elmira College					x	x				
Fisk University							x			
Fordham University								x	x	x
Frick Educational Comm.									x	
General Education Board	x	x	x	x	x					
Georgia, Univ. of Medical				x						
Goucher College	x	x	x	x	x	x	x	x	x	x
Hamilton College		x	x	x		x		x		
Harvard University	x	x	x	x	x	x	x	x	x	x
Harvard University Medical	x	x	x	x	x	x	x	x	x	x
Heidelberg College									x	
Howard University						x				
Hunter College	x	x	x	x	x	x	x		x	
Illinois, University of	x	x	x	x	x	x	x	x	x	x
Indiana University								x		
Industrial and Engin. Chem.	x	x	x	x	x	x	x	x	x	x
Iowa, State Univ. of	x	x	x	x	x	x	x	x	x	x
Iowa, State College of	x	x	x	x	x	x				
Johns Hopkins University	x	x	x	x	x	x	x	x	x	x
Johns Hopkins University Medical	x					x	x			
Johnson Foundation			x							x
Josiah Macy Foundation								x		
Julius Rosenwald Fund									x	
Kansas, University of	x		x				x			
Kenyon College				x	x					
Leland Stanford Univ.							x			
Lincoln University		x								
Long Island University	x	x	x	x	x	x	x		x	
Loyala Univ. Medical								x		
McGill University						x				
Markle Foundation									x	
Maryland, Univ. of Medical			x	x	x	x		x	x	x
Marine Studios, Inc.									x	
Mass. General Hospital										x
Mass. State College				x	x	x	x	x	x	
Memorial Hospital, N. Y.		x	x	x	x	x				
Michigan, University of								x		
Minnesota, University of			x	x	x					
Missouri, University of		x	x		x	x	x	x	x	
Morehouse College	x	x	x	x		x			x	

EXHIBIT 5—*Continued*

	1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Mt. Holyoke College	x	x	x	x	x	x	x	x	x	x
Mt. Sinai Hospital							x	x	x	
Mundelein University						x		x		
National Research Council									x	
N. Y. Dept. of Health	x	x	x	x	x	x	x	x	x	
New York University	x	x	x	x	x	x	x	x	x	x
New York University Medical	x	x	x	x	x		x	x	x	x
New York University Wash. Square	x	x	x	x	x	x	x	x	x	x
N. Carolina Coll. for Negroes							x			
Northwestern University	x	x	x	x	x	x	x	x		
Notre Dame University					x	x				
Oberlin College	x	x	x	x	x	x	x	x	x	x
Ohio State University							x	x	x	x
Ohio Wesleyan University								x		
Penn. College for Women	x	x	x	x	x	x	x		x	x
Pennsylvania, Univ. of	x	x	x	x	x	x	x	x	x	x
Pennsylvania, Univ. of Medical	x	x	x	x	x	x	x	x	x	x
Pittsburgh, Univ. of	x	x	x	x	x	x	x	x	x	
Princeton University	x	x	x	x	x	x	x	x	x	x
Purdue University		x	x	x	x	x	x	x		
Radcliffe College	x	x	x	x	x	x	x	x	x	x
Rensselaer Poly. Inst.			x							
Rice Institute				x						
Rochester, University of	x	x	x	x	x	x	x	x	x	x
Rochester, University of Medical	x	x		x	x					
Rockefeller Foundation	x	x	x	x	x	x	x			
Rockefeller Institute	x	x	x	x	x	x	x	x	x	x
Royal Egyptian Foundation		x								
Russell Sage College							x	x	x	
Rutgers University	x		x	x	x	x	x	x	x	x
St. Elizabeth, College of	x									
St. Francis Xavier College			x							
St. Johns College		x		x	x	x				
Sarah Lawrence College								x		
Seton Hall College		x	x							
Seton Hill College				x				x		x
Smith College	x	x	x	x	x	x	x	x	x	x
J. C. Smith University	x	x								
Spring Hill College					x					
Springfield College							x	x		
Swarthmore College	x	x	x	x	x					
Sweet Briar College									x	

EXHIBIT 5—*Continued*

	1934	'35	'36	'37	'38	'39	'40	'41	'42	'43
Syracuse University	x	x	x	x	x		x	x	x	
Syracuse University Medical						x	x			
Temple University	x	x	x	x	x	x				
Toledo, University of					x	x		x		
Tufts College	x	x	x		x	x	x	x	x	x
Tulane University								x		
Tulane University Newcomb Coll.	x							x		
Union College, N. Y.		x	x	x	x			x	x	
Union College, Ky.						x	x			
U. S. Fish and Wild Life Serv.										x
Vanderbilt University		x								
Vanderbilt University Medical	x	x	x	x	x	x	x	x	x	
Vassar College	x	x	x	x	x	x	x	x	x	x
Vermont, University of		x			x					
Villanova College						x	x	x	x	
Virginia, University of	x	x	x	x	x	x	x	x		
Wabash College	x	x	x	x	x		x	x		
Washington University				x	x	x	x	x	x	x
Washington University Medical				x	x	x	x	x		
Wellesley College	x	x	x	x	x	x	x	x	x	x
Wesleyan University	x	x	x	x	x	x	x	x	x	
Western Reserve University		x			x	x				
Wheaton College	x	x	x	x	x	x	x	x	x	x
William and Mary College					x	x				
Williams College					x	x				
Wilson College	x	x	x	x	x	x	x			
Wisconsin, University of		x	x	x						
Wistar Institute	x	x	x	x						
Woods Hole Oceanographic Inst.									x	x
Yale University	x	x	x	x	x	x	x	x	x	x
Yale University Medical							x	x		

EXHIBIT 6

Additions to the Library

	Bound volumes	Reprints	Sets completed	Partially completed	New journals	Classics	Budget
1934	1,138	5,028	8	16	18		$20.325
1935	1,622	4,478	24	17	55		22.444
1936	2,107	3,339	29	12	28		22.510
1937	1,155	7,042	24	20	21		22.029
1938	1,455	6,905	33	30	15		19.515
1939	1,239	3,850	33	24	21		22.149
1940	1,561	3,528	20	46	21		17.923
1941	1,482	3,321	24	91	25	4	16.964
1942	1,758	3,097	45	161	13	23	15.332
1943	1,008	7,927	10	51	10	5	11.047
Total added	14,525	48,515	250	468	227	32	
Total in Libr.	51,945	129,723	1800 approx.	600 approx.			

4. THE STAFF, 1944

Charles Packard, Director, Marine Biological Laboratory, Woods Hole, Massachusetts.

Senior Staff of Investigation

E. G. Conklin, Professor of Zoology, Emeritus, Princeton University.
Frank R. Lillie, Professor of Embryology, Emeritus, The University of Chicago.
Ralph S. Lillie, Professor of General Physiology, Emeritus, The University of Chicago.
C. E. McClung, Professor of Zoology, Emeritus, University of Pennsylvania.
S. O. Mast, Professor of Zoology, Emeritus, Johns Hopkins University.
A. P. Mathews, Professor of Biochemistry, Emeritus, University of Cincinnati.
T. H. Morgan, Director of the Biological Laboratory, California Institute of Technology.
G. H. Parker, Professor of Zoology, Emeritus, Harvard University.

ZOOLOGY

I. Consultants

T. H. Bissonnette, Professor of Biology, Trinity College.
L. L. Woodruff, Professor of Protozoology, Yale University.

II. Instructors

J. B. Buck, Assistant Professor of Zoology, University of Rochester, in charge of course.
T. H. Bullock, Instructor in Neuro Anatomy, Yale University.
W. D. Burbanck, Associate Professor of Biology, Drury College.
C. G. Goodchild, Associate Professor of Biology, Southwest Missouri State Teachers College.
John H. Lochhead, Instructor in Zoology, University of Vermont.
Madelene E. Pierce, Assistant Professor of Zoology, Vassar College.
W. M. Reid, Assistant Professor of Biology, Monmouth College.
Mary D. Rogick, Professor of Biology, College of New Rochelle.

III. Laboratory Assistants

Gene Lehman, Teaching Fellow Zoology, University of North Carolina.
Mary E. Banks, Washington University.

EMBRYOLOGY

I. Consultants

L. G. Barth, Assistant Professor of Zoology, Columbia University.
H. B. Goodrich, Professor of Biology, Wesleyan University.

II. Instructors

Viktor Hamburger, Professor of Zoology, Washington University, in charge of course.
Donald P. Costello, ~~Assistant~~ Professor of Zoology, University of North Carolina.
Ray L. Watterson, Assistant Professor of Zoology, University of California.

PHYSIOLOGY

I. Consultants

William R. Amberson, Professor of Physiology, University of Maryland, School of Medicine.
Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
Merkel H. Jacobs, Professor of Physiology, University of Pennsylvania.

II. Instructors

Arthur K. Parpart, Associate Professor of Biology, Princeton University, in charge of course.
Robert Ballentine, Lecturer in Zoology, Columbia University.
Arthur C. Giese, Associate Professor of Biology, Stanford University (absent in 1943).
Rudolf T. Kempton, Professor of Zoology, Vassar College.

BOTANY

I. Consultants

S. C. Brooks, Professor of Zoology, University of California.
B. M. Duggar, Professor of Plant Physiology, University of Wisconsin.

II. Instructors

Wm. Randolph Taylor, Professor of Botany, University of Michigan, in charge of course.
Hannah Croasdale, Technical Assistant, Dartmouth College.

EXPERIMENTAL RADIOLOGY

G. Failla, Memorial Hospital, New York City.
L. Robinson Hyde, Phillips Exeter Academy, Exeter, N. H.

LIBRARY

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian
Deborah Lawrence   Mrs. Elon H. Jessup   Mary A. Rohan

APPARATUS DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager
J. D. Graham

CHEMICAL DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager

SUPPLY DEPARTMENT

James McInnis, Manager
Ruth Crowell
Grace M. Kennerson
M. B. Gray
W. E. Kahler
G. Lehy
A. M. Hilton
A. W. Leathers
F. N. Whitman

GENERAL OFFICE

F. M. MacNaught, Business Manager
Polly L. Crowell
Mrs. Carlotta I. Cowin

GENERAL MAINTENANCE

T. E. Larkin, Superintendent
W. C. Hemenway
G. T. Nickelson, Jr.
R. W. Kahler
T. E. Tawell
A. J. Pierce

THE GEORGE M. GRAY MUSEUM

George M. Gray, Curator Emeritus

---

5. INVESTIGATORS AND STUDENTS

Independent Investigators, 1944

Abell, Richard G., Assistant Professor of Anatomy, University of Pennsylvania.
Addison, William H. F., Professor of Normal Histology and Embryology, University of Pennsylvania.
Allee, W. C., Professor of Zoology, University of Chicago.
Anderson, Rubert S., Assistant Professor, University of Maryland.
Ballentine, Robert, Instructor, Columbia University.
Barth, L. G., Assistant Professor of Zoology, Columbia University.
Bartlett, James H., Associate Professor of Theoretical Physics, University of Illinois.
Belcher, Jane C., Instructor, Sweet Briar College.
Berger, Charles A., Director, Biological Laboratory, Fordham University.
Bergmann, Werner, Associate Professor, Yale University.
Bertholf, Lloyd Millard, Dean of Faculty and Professor of Biology, Western Maryland College.
Brown, Dugald S., Professor of Physiology, New York University.
Brownell, Katharine A., Research Associate, Ohio State University.
Buck, John B., Assistant Professor of Zoology, University of Rochester.
Budington, Robert A., Professor of Zoology, Emeritus, Oberlin College.
Bullock, Theodore H., Instructor in Neuro-Anatomy, Yale University.
Burbanck, W. D., Associate Professor of Biology and Chairman of Department, Drury College.

Chambers, Robert, Research Professor of Biology, New York University.
Clark, Eleanor L., Voluntary Research Worker, University of Pennsylvania, Medical School.
Clark, Eliot R., Professor and Head of Department of Anatomy, University of Pennsylvania, Medical School.
Clement, A. C., Associate Professor of Biology, College of Charleston.
Clowes, G. H. A., Director of Research, Lilly Research Laboratories, Eli Lilly and Company.
Conklin, Edward G., Professor Emeritus of Biology, Princeton University.
Copeland, Manton, Professor of Biology, Bowdoin College.
Costello, Donald P., Professor of Zoology, University of North Carolina.
Crampton, H. E., American Museum of Natural History.
Croasdale, Hannah T., Technical Assistant in Zoology, Dartmouth College.
Cutkomp, Laurence K., Research Fellow in Zoology, University of Pennsylvania.
Elliott, S. D., Visiting Investigator, Rockefeller Institute.
Failla, G., Professor of Radiology, College of Physicians and Surgeons, Columbia University.
Froehlich, Alfred, Associate, May Institute for Medical Research, Cincinnati, Ohio.
Galtsoff, Paul S., Senior Biologist, U. S. Fish and Wildlife Service.
Garrey, W. E., Professor of Physiology, Vanderbilt University, School of Medicine.
Giese, Arthur C., Associate Professor of Biology, Stanford University of California.
Glaser, Otto C., Professor of Biology, Amherst College.
Goodchild, C. G., Associate Professor of Biology, State Teachers College.
Gorbman, Aubrey, Instructor in Biology, Wayne University.
Grand, C. G., Research Associate, New York University.
Grell, Sister Mary, Student and Investigator, Fordham University, New York.
Hallock, Frances A., Associate Professor, Hunter College.
Hamburger, Viktor, Professor of Zoology, Washington University.
Harnly, Morris H., Associate Professor, Washington Square College, New York University.
Harris, Daniel L., Research Associate, University of Pennsylvania.
Hartman, Frank A., Professor and Chairman of Department of Physiology, Ohio State University.
Harvey, E. Newton, Professor of Physiology, Princeton University.
Harvey, Ethel Browne, Independent Investigator, Princeton University.
Heilbrunn, L. V., Professor of Zoology, University of Pennsylvania.
Hiatt, Edwin P., Assistant Professor of Physiology, New York University.
Hopkins, Hoyt S., Associate Professor of Physiology, New York University, Dental College.
Jacobs, M. H., Professor of General Physiology, University of Pennsylvania, Medical School.
Kempton, Rudolf T., Professor of Zoology, Vassar College.
Knowlton, Frank P., Professor of Physiology, College of Medicine, Syracuse University.
Krahl, Maurice E., Research Biological Chemistry, Lilly Research Laboratories.
Lancefield, Rebecca C., Associate Member, Rockefeller Institute for Medical Research.
Lavin, George I., In Charge of Spectroscopic Laboratory, Rockefeller Institute for Medical Research.
Lazarow, Arnold, Senior Instructor, Western Reserve University.
Levy, Milton, Assistant Professor, New York University College of Medicine.
Lewis, Margaret Reed, Research Associate, The Carnegie Institution of Washington.
Lewis, Warren H., Member, The Wistar Institute of Anatomy and Biology.
Liebman, Emil, Research Fellow, Princeton University.
Lillie, Ralph S., Professor Emeritus of Physiology, University of Chicago.
Little, Elbert P., Instructor in Science, Exeter Academy, Exeter, New Hampshire.
Lochhead, John H., Instructor in Zoology, University of Vermont.
McClung, C. E., Professor of Zoology, Emeritus, University of Pennsylvania.
McElroy, William D., Research Associate, Princeton University.
MacLean, Bernice L., Assistant Professor, Department of Biological Sciences, Hunter College.
McMaster, Philip D., Associate Member, Rockefeller Institute.
Marsland, Douglas A., Associate Professor, New York University, Washington Square College.
Mast, S. O., Professor of Zoology, Emeritus, Johns Hopkins University.
Mathews, Albert P., Professor of Biochemistry, Emeritus, University of Cincinnati.

Memhard, Allen R., Crescent Road, Riverside, Connecticut.
Metz, Charles B., Instructor in Biology, Wesleyan University.
Metz, C. W., Director of Zoological Laboratory, Chairman of Department of Zoology, University of Pennsylvania.
Michaelis, Leonor, Member Emeritus, Rockefeller Institute for Medical Research.
Nachmansohn, David, Research Associate in Neurology, Columbia University.
Oppenheimer, Jane M., Assistant Professor of Biology, Bryn Mawr College.
Osterhout, W. J. V., Member Emeritus, Rockefeller Institute for Medical Research.
Parker, George H., Professor of Zoology, Emeritus, Harvard University.
Parpart, Arthur K., Associate Professor, Princeton University.
Pierce, Madelene E., Assistant Professor of Zoology, Vassar College.
Pollister, Arthur W., Associate Professor of Zoology, Columbia University.
Reid, W. Malcolm, Assistant Professor of Biology, and Department Head, Monmouth College.
Renn, Charles E., Associate Sanitary Biologist, Massachusetts Department of Health.
Reynolds, J. Paul, Professor of Biology, Birmingham Southern College.
Richards, A. Glenn, Jr., Instructor in Zoology, University of Pennsylvania.
Rogick, Mary Dora, Professor of Biology, College of New Rochelle, New Rochelle, New York.
Rugh, Roberts, Associate Professor of Biology, New York University.
Schaeffer, A. A., Professor of Biology, Temple University.
Schallek, William B., Teaching Fellow, Harvard University.
Scharrer, Ernst A., Assistant Professor of Anatomy, Western Reserve University School of Medicine.
Schmitt, Francis O., Professor of Biology, Massachusetts Institute of Technology.
Scott, Sister Florence Marie, Professor of Biology, Seton Hill College.
Shapiro, Harry H., Assistant Professor, Department of Anatomy, College of Physicians and Surgeons, Columbia University.
Sichel, F. J., Associate Professor of Physiology, University of Vermont, College of Medicine.
Steinbach, H. B., Associate Professor of Zoology, Washington University.
Stewart, Dorothy R., Research Fellow in Physiology, University of Pennsylvania.
Stokey, Alma G., Professor Emeritus of Plant Science, Mount Holyoke College.
Stunkard, Horace W., Professor of Biology, Head of Department, New York University.
Taft, Charles H., Associate Professor of Pharmacology, Medical Branch, University of Texas.
Taylor, William Randolph, Professor of Botany, University of Michigan.
TeWinkel, Lois E., Associate Professor of Zoology, Smith College.
Troedsson, Pauline H., Instructor in Biology, Brooklyn College.
Wainio, Walter W., Assistant Professor of Physiology, New York University.
Watterson, Ray L., Assistant Professor of Zoology, University of California.
Wenrich, D. H., Professor of Zoology, University of Pennsylvania.
Whiting, Anna R., Associate Professor of Zoology, Swarthmore College.
Whiting, P. W., Associate Professor of Zoology, University of Pennsylvania.
Wichterman, Ralph, Assistant Professor, Temple University.
Witkus, Eleanor Ruth, Instructor in Botany and Bacteriology, Fordham University.
Wrinch, Dorothy, Lecturer in Physics, Smith College.
Yntema, Chester L., Assistant Professor of Anatomy, Cornell University, Medical College.
Zweifach, Benjamin W., Research Associate in Biology, New York University.

Beginning Investigators, 1944

Edelman, Abraham, Graduate Student, Columbia University.
Hermanson, Virginia, Graduate Student, Ohio State University.
Jaeger, Lucena, Graduate Student, Columbia University.
Keister, Margaret L., Instructor, Wheaton College.
Koopman, Karl F., Graduate Student, Columbia University.
Krugelis, Edith J., Research Assistant and Graduate Student, Columbia University.
Lehman, Gene, Teaching Fellow, University of North Carolina.
Low, Ruth H., Graduate Student, Zoology Department, Columbia University.
Mortensen, Edith, Assistant Professor of Zoology, The George Washington University.

Schneyer, Leon H., Instructor, New York University College of Dentistry.
Sisselman, Charlotte B., Research Student, Columbia University.

Library Readers, 1944

Amberson, William R., Professor of Physiology, University of Maryland.
Anderson, Thomas F., Associate, Johnson Foundation, University of Pennsylvania.
Armstrong, Philip B., Professor of Anatomy, College of Medicine, Syracuse University.
Bevelander, Gerrit, Associate Professor of Anatomy, New York University.
Bissonnette, T. Hume, Professor of Biology, Trinity College.
Bodian, David, Associate in Epidemiology, Johns Hopkins University.
Cahen, Raymond L., Research Assistant, Yale University, Medical School.
Cassidy, Harold G., Assistant Professor of Chemistry, Yale University.
Choucroun, Nine, Cornell University Medical College.
Clarke, Robert W., Research Assistant, Yale University, Medical School.
Cox, Edward H., Professor of Chemistry, Swarthmore College.
Dische, Zacharious, Department of Biochemistry, College of Physicians and Surgeons, Columbia University.
Faust, Ernest C., Professor of Parasitology and Director Department of Tropical Medicine, Tulane University.
Fridemann, Ulrich, Chief of Division of Bacteriology, Brooklyn Jewish Hospital.
Furth, Jacob, Associate Professor of Pathology, Cornell University Medical College.
Gates, R. Ruggles, Emeritus Professor, University of London.
Goodrich, H. B., Professor of Biology, Wesleyan University.
Gurewich, Vladimir, Assistant Visiting Physician, New York College of Medicine and Bellevue Hospital.
Haywood, Charlotte, Professor of Physiology, Mount Holyoke College.
Johlin, J. M., Associate Professor of Biochemistry, Vanderbilt University.
Keller, Rudolph, Prague, Czechoslovakia.
Keyes, Donald B., Professor of Chemical Engineering, University of Illinois.
Krasnow, Frances, Head of Department of Biological Chemistry-Related Basic Sciences, Guggenheim Dental Clinic.
Landis, Eugene M., Professor of Physiology, Harvard University, Medical School.
Lee, Richard E., Research Assistant, Columbia University, College of Physicians and Surgeons.
Loewi, Otto, Research Professor, New York University.
Mavor, James W., Provessor of Biology, Union College.
Menkin, Valy, (Fellow), Guggenheim Research Foundation.
Meyerhof, Otto, Research Professor of Biochemistry, University of Pennsylvania.
Moldaver, Joseph, Research Associate in Neurology, Columbia University.
Moschowitz, Eli, Assistant Professor of Chemical Medicine, Columbia University.
O'Brien, John A., Jr., Instructor in Biology, Catholic University of America.
Ochoa, Severo, Research Associate in Medicine, New York University, College of Medicine.
Oseasohn, Robert, Student, Long Island College of Medicine.
Perlmanm, Gertrude E., Research Fellow, Harvard University, Medical School.
Powdermaker, Hortense, Assistant Professor of Anthropology, Queens College.
Runner, Meredith, Instructor, University of Connecticut.
Shen, Shih-Chang, Member, National Institute of Physiology, China.
Shwartzman, Gregory, Head Bacteriologist, The Mount Sinai Hospital.
Singer, Marcus, Harvard University, Medical School.
Smelser, George K., Assistant Professor of Anatomy, Columbia University.
Speidel, Carl C., Professor of Anatomy, University of Virginia.
Stern, Kurt G., Chief Research Chemist, Overly Biochemical Research Foundation, New York City.
Tashiro, Kiyoshi, University of Cincinnati, College of Medicine.
Wagner, Carroll E., Research Assistant, Histology, Naval Medical Research Institute.
Weidenreich, Franz, Honorary Director, Cenozoic Research Laboratory, China.
Weiner, Nathan, Director of Research, Endo Products, Inc.

Willier, B. H., Professor of Zoology, Director of Biological Laboratories, The Johns Hopkins University.
Woodward, Alvalyn E., Assistant Professor, University of Michigan.
Zorzoli, Anita, Teaching Fellow, New York University.

Research Assistants, 1944

Abramsky, Tessie, Technician, Rockefeller Institute for Medical Research.
Brunelli, Eleanor L., Research Assistant, New York University Dental School.
DeFalco, Rose H., Research Assistant-Secretary, University of Pennsylvania.
Dziorney, Leon, Research Assistant, New York University.
Frumin, M. R., Research Assistant, University of Pennsylvania.
Goldis, Bernice R., Research Assistant, University of Pennsylvania.
Gregg, John R., Graduate Student, Princeton University.
Hirst, Shirley M., Research Assistant, University of Pennsylvania.
Honegger, Carol, Temple University.
Hopkins, Amos, Junior Engineering Aide, Massachusetts State Health Department.
Lawler, H. Claire, Research Assistant, New York University.
LeFevre, Linda, Research Assistant, University of Pennsylvania.
LeFevre, Paul G., Research Assistant, University of Pennsylvania.
Levy, Betty, Laboratory Technician, Rockefeller Institute.
Marks, Mildred H., Assistant Research Worker, University of Pennsylvania.
Morton, Jane W., Technical Assistant in Zoology, University of Pennsylvania.
Price, Winston Harvey, Research Assistant, University of Pennsylvania.
Quinn, Gertrude P., Research Assistant, New York University.
Wilson, Walter L., Research Associate, University of Pennsylvania.
Woodward, Arthur A., Research Assistant, University of Pennsylvania.

Students, 1944

BOTANY

Chew, Robert M., Student, Washington & Jefferson College.
Devine, Verona, Student, Hunter College.
Guzman, Julia, Student, Washington University.
Hoskins, Barbara, Student, Wellesley College.
Mittlacher, Helen, Student, Wheaton College.

EMBRYOLOGY

Anderson, Joan C., Student, McGill University.
Courant, Gertrude E., Student, Swarthmore, College.
Cullen, Sister Mary Urban, Graduate Student, Yale University.
Davidson, Margaret E. M., Student, McGill University.
Farfante, Isabel Perez, Student, Cambridge, Massachusetts.
Finkelstein, Grace, Teaching Fellowship, New York University.
Getz, Charlotte E., Undergraduate Student, University of Chicago.
Godwin, Doris Ruth, Graduate Assistant, University of North Carolina.
Henley, Catherine, Graduate Assistant, University of North Carolina.
Honegger, Carol Marie, Student, Temple University.
Kelley, Ellen Mary, Student, New Jersey College for Women.
Kivy, Evelyn, Instructional Staff, Brooklyn College.
Landau, Carol, Student, Goucher College.
Lantz, Elsie Jean, Student, Washington University.
McGovern, Beulah H., Teaching Fellow, New York University.
Murray, Helen Ernestine, Student, Emmanuel College.
Potts, Ella Elizabeth, Student, Sarah Lawrence College.
Roth, Owen H., Instructor in Biology, St. Vincent College.

Schneller, Sister Mary Beatrice, Professor, Saint Joseph College for Women.
Strong, Helen Margaret, Teaching Fellow, Smith College.
Vishniac, Wolf, Student, Brooklyn College.
Williamson, Frances Alice, Student, New Jersey College for Women.
Willis, Marian, Student, Iowa State College.

PHYSIOLOGY

Bernstein, Jeane, Graduate Student, New York University.
Carson, Gweneth, Student, University of Toronto.
Cregar, Mary, Demonstrator in Physiology, Bryn Mawr College.
Keister, Margaret Louise, Instructor in Zoology, Wheaton College.
McLean, Dorothy Juanita, Graduate Student, University of Toronto.
Partridge, Judith Ann, Assistant in Physiology, Vassar College.
Pepper, Billie Barbara, Student, Radcliffe College.
Reich, Eva, Student, Barnard College.
Taylor, Babette, Student, Washington University.
Therien, Mercedes, Assistant Research, Montreal University.

ZOOLOGY

Austin, Jane, Student, Randolph-Macon Woman's College.
Banks, Mary Elizabeth, Research Assistant, Washington University.
Barrows, Shirley Louise, Student, University of Rochester.
Benson, Eleanore Bie, Student, University of Pennsylvania.
Butt, Ferdinand H., Instructor, Cornell University.
Calkins, Janet Elizabeth Morse, University of Chicago.
Conravey, June Rose, Student Assistant, Newcomb College, Tulane University.
deRevere, Joan Brooks, Undergraduate Student, Wilson College.
Douglis, Marjorie B., Assistant in Zoology, Chicago University.
Dunbar, Sally, De Pauw University.
Fairfield, Janet, Student, Russell Sage College.
Falkner, Etta, Instructor, American Museum of Natural History.
Fogerson, Virginia Lee, Drury College.
Gosford, Barbara, Duke University.
Habert, Yvonne A., High School Teacher, City of Boston.
Koopman, Karl Friedrich, Graduate Student, Columbia University.
Langman, Ida K., University of Pennsylvania.
Lauthers, Rosemary Ann, Student, Oberlin College.
Leduc, Elizabeth Hortense, Graduate Assistant, Wellesley College.
Lloyd, Mary Remsen, Vassar College.
Marks, Mildred Helen, Graduate Student, University of Pennsylvania.
McClintock, Mary, Instructor, Bemidji, Minnesota.
Neal, Lucy Lee, Drury College.
Randall, Nancy Lois, Student, Swarthmore College.
Reese, Jean, Goucher College.
Root, Oscar M., Instructor, Brooks School.
Roth, Owen Harold, Instructor in Biology, St. Vincent College.
Schmid, Leo A., Baltimore, Md.
Slavin, Alice Cecilia, Student, Seton Hill College.
Southwell, Violet M., Student, Wilson College.
Steenburg, Isabella, Student, Vassar College.
Stekl, Eleanor B., Science Teacher, N. Tonawanda High School.
Sweeney, Patricia Georgia, Student, Oberlin College.
Van Geyt, Virginia, Student, University of Rochester.
Viosca, Miriam A., Student Assistant, Newcomb College, Tulane University.
Weller, Doris A., Undergraduate, Radcliffe College.
Warner, Rose Ella, Teacher of Biology, Frick Educational Commission.

6. TABULAR VIEW OF ATTENDANCE

	1940	*1941*	*1942*	*1943*	*1944*
Investigators—Total	386	337	201	160	193
Independent	253	197	132	89	112
Under instruction	62	59	16	19	11
Library readers	—	31	28	35	50
Research assistants	71	50	25	17	20
Students—Total	128	131	74	68	75
Zoology	55	55	36	47	37
Protozoology (not given after 1940)	7	—	—	—	—
Embryology	34	37	24	13	23
Physiology	22	24	6	8	10
Botany	10	15	8	—	5
Total Attendance	514	468	275	228	276
Less persons registered as both students and investigators	7	7	2	6	1
	507	461	273	222	275
Institutions Represented—Total	148	144	126	116	106
By investigators	112	102	83	70	74
By students	79	72	43	41	41
Schools and Academies Represented					
By investigators	1	5	2	2	1
By students	2	2	—	1	2
Foreign Institutions Represented					
By investigators	2	3	—	2	2
By students	1	1	—	—	3

7. SUBSCRIBING AND COOPERATING INSTITUTIONS

1944

Amherst College
Barnard College
Bowdoin College
Brooklyn College
Bryn Mawr College
Catholic University of America
Columbia University
Cornell University
Cornell University Medical College
Duke University
Fish and Wild Life Service, U. S. Dept. of the Interior
Fordham University
Henry C. Frick Educational Commission
Goucher College
Harvard University
Hunter College
Industrial and Engineering Chemistry, of the American Chemical Society
Johns Hopkins University
The Lankenau Hospital Research Institute
Eli Lilly and Co.
Massachusetts Department of Health
Massachusetts Institute of Technology
Mount Holyoke College
H. Sophie Newcomb College
New York University
New York University College of Medicine
New York University Washington Square College
Oberlin College
Ohio State University
Princeton University
Radcliffe College
Rockefeller Institute for Medical Research
Russell Sage College
St. Joseph College for Women
Smith College
State University of Iowa
Syracuse University
Syracuse University Medical School
Temple University
Tufts College
University of Chicago
University of Cincinnati
University of Illinois
University of Maryland Medical School
University of Pennsylvania
University of Pennsylvania School of Medicine
University of Rochester

Vassar College
Villanova College
Washington University
Wayne University
Wellesley College
Wesleyan University
Western Reserve University
Wheaton College
Wilson College
Wistar Institute
Woods Hole Oceanographic Institution
Yale University

8. EVENING LECTURES, 1944

Friday, June 30	Dr. T. H. Bissonnette	"Some Recent Studies on Photoperiodicity in Animals, particularly Fur-bearers."
Friday, July 7	Dr. Ethel Browne Harvey	"Some Results of Centrifuging the Arbacia Egg."
Friday, July 14	Dr. A. C. Giese	"Ultraviolet Radiations and the Life Activities of Cells."
Friday, July 21	Dr. H. J. Muller	"Evidence for the Meticulousness of Adaptation."
Friday, July 28	Dr. Carl C. Speidel	"Experimental Studies of Special Sensory Organs and Nerves."
Thursday, August 3	Dr. Ernest Carroll Faust	"Problems of Tropical Medicine in the United States."
Friday, August 4	Dr. A. K. Parpart	"Blood Preservation: A Problem in Cellular Physiology."
Thursday, August 10	Mr. G. G. Lower	"Local Invertebrates."
Friday, August 11	Dr. W. C. Allee	"Social Orders Among Vertebrates."
Wednesday, August 16	Dr. Ralph Turner	"Rehabilitation of Scientific Institutions in Devastated Europe."
Friday, August 18	Dr. A. W. Pollister	"The Centriole Problem."
Friday, August 25	Prof. G. H. Parker	"Animal Coloration, Fixed and Changeable."

9. SHORTER SCIENTIFIC PAPERS, 1944

Tuesday, July 18	Dr. B. H. Willier	"Melanophore Control of Sexual Dimorphism in Feather Pigmentation of the Barred Rock Fowl."
	Dr. Viktor Hamburger	"The Effects of Peripheral Factors on Motor Neuron Differentiation in the Chick Embryo."
	Dr. W. H. Lewis	"The Superficial Gel Layer and Its Role in Development."

Tuesday, July 25

Dr. Leonor Michaelis	"Ferritin and Iron Metabolism."
Dr. Arnold Lazarow	"The Chemical Organization of the Cytoplasm of the Liver Cell."
Dr. Leonor Michaelis	"Theory of Metachromatic Staining."

Tuesday, August 1

Dr. Dorothy Wrinch	"The Native Protein in Crystalline Form."
Dr. Otto Myerhof	"The Role of Adenylpyro-Phosphatase in Alcoholic Fermentation of Yeast."
Dr. Ernest Scharrer	"The Naples Station Still Lives."

Tuesday, August 8

Dr. A. M. Shanes	"Application of Bio-electricity to the Study of Functioning in Nerve."
Dr. David Nachmansohn	"On the Energy Source of the Nerve Action Potential."
Dr. T. H. Bullock	"Oscillographic Studies on the Giant Nerve Fiber System in Lumbricus."
Dr. Paul Weiss	"Evidence for the Perpetual Proximo-distal Growth of Nerve Fibers."

Tuesday, August 15

Dr. L. V. Heilbrunn Dr. D. L. Harris Dr. P. G. LeFevre Dr. W. H. Price Dr. W. L. Wilson Dr. A. A. Woodward, Jr.	"A Toxic Substance from Protoplasm."
Dr. D. L. Harris Dr. W. H. Price Dr. L. V. Heilbrunn	"The Chemical Nature of a Toxic Substance from Protoplasm."
Dr. G. I. Lavin	"Recent Developments in Ultraviolet Microscopy."

Tuesday, August 22

Dr. B. W. Zweifach	"The Peripheral Circulation in Traumatic Shock."
Dr. W. R. Amberson	"Recent Experience with Hemoglobin-saline Solutions."
Dr. R. G. Abell Dr. W. M. Parkins	"Gelatin as a Plasma Substitute."

Thursday, August 24

Dr. C. A. Berger	"Experimental Studies on the Cytology of Allium."
Dr. Valy Menkin	"Studies on the Chemical Basis of Fever."
Dr. Miriam F. Menkin	"In Vitro Fertilization of Human Ova."

Tuesday, August 29

Dr. L. M. Bertholf	"Studies on Metamorphosis in the Tunicate."
E. Mortensen Dr. P. S. Galtsoff	"Behavior and Tube Building Habits of Polydora ligni."
Dr. J. B. Buck	"The Click Mechanism of Elaterid Beetles."

Thursday, August 31

Dr. G. K. Smelser .... "Orbital Changes in Experimental Exophthalmos."

Dr. A. Gorbman .... "Radioactive Iodine Absorption in Lower Chordates and the Problem of Homology of the Thyroid Gland."

Dr. D. L. Harris .... "Phosphoprotein Phosphatase, a New Enzyme from the Frog Egg."

10. MEMBERS OF THE CORPORATION, 1944

1. Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Maryland.
Beckwith, Dr. Cora J., Vassar College, Poughkeepsie, New York.
Billings, Mr. R. C., 66 Franklin Street, Boston, Massachusetts.
Calvert, Dr. Philip P., University of Pennsylvania, Philadelphia, Pennsylvania.
Cole, Dr. Leon J., College of Agriculture, Madison, Wisconsin.
Conklin, Prof. Edwin G., Princeton University, Princeton, New Jersey.
Cowdry, Dr. E. V., Washington University, St. Louis, Missouri.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Massachusetts.
Foot, Miss Katherine, Care of Morgan Harjes Cie, Paris, France.
Jackson, Mr. Chas. C., 24 Congress Street, Boston, Massachusetts.
Jackson, Miss M. C., 88 Marlboro Street, Boston, Massachusetts.
King, Mr. Chas. A.
Kingsbury, Prof. B. F., Cornell University, Ithaca, New York.
Lewis, Prof. W. H., Johns Hopkins University, Baltimore, Maryland.
Means, Dr. J. H., 15 Chestnut Street, Boston, Massachusetts.
Moore, Dr. George T., Missouri Botanical Gardens, St. Louis, Missouri.
Moore, Dr. J. Percy, University of Pennsylvania, Philadelphia, Pa.
Morgan, Mrs. T. H., Pasadena, California.
Morgan, Prof. T. H., Director of Biological Laboratory, California Institute of Technology, Pasadena, California.
Noyes, Miss Eva J.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pennsylvania.
Scott, Dr. Ernest L., Columbia University, New York City, New York.
Sears, Dr. Henry F., 86 Beacon Street, Boston, Massachusetts.
Shedd, Mr. E. A.
Thorndike, Dr. Edward L., Teachers College, Columbia University, New York City, New York.
Treadwell, Prof. A. L., Vassar College, Poughkeepsie, New York.
Trelease, Prof. William, University of Illinois, Urbana, Illinois.
Waite, Prof. F. C., 144 Locust Street, Dover, New Hampshire.
Wallace, Louise B., 359 Lytton Avenue, Palo Alto, California.

2. Regular Members

Adams, Dr. A. Elizabeth, Mount Holyoke College, South Hadley, Massachusetts.
Addison, Dr. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.

Adolph, Dr. Edward F., University of Rochester Medical School, Rochester, New York.

Albaum, Dr. Harry G., 3115 Avenue I, Brooklyn, New York.

Albert, Dr. Alexander, Biological Laboratories, Harvard University, Cambridge, Massachusetts.

Allee, Dr. W. C., The University of Chicago, Chicago, Illinois.

Amberson, Dr. William R., Department of Physiology, University of Maryland, School of Medicine, Lombard and Greene Streets, Baltimore, Maryland.

Anderson, Dr. Rubert S., University of Maryland School of Medicine, Department of Physiology, Baltimore, Maryland.

Anderson, Dr. T. F., University of Pennsylvania, Philadelphia, Pennsylvania.

Armstrong, Dr. Philip B., College of Medicine, Syracuse University, Syracuse, New York.

Austin, Dr. Mary L., Wellesley College, Wellesley, Massachusetts.

Baitsell, Dr. George A., Yale University, New Haven, Connecticut.

Baker, Dr. H. B., Zoological Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania.

Ballard, Dr. William W., Dartmouth College, Hanover, New Hampshire.

Ballentine, Dr. Robert, Columbia University, Department of Zoology, New York City, New York.

Ball, Dr. Eric G., Department of Biological Chemistry, Harvard University Medical School, Boston, Massachusetts.

Bard, Prof. Philip, Johns Hopkins Medical School, Baltimore, Maryland.

Barron, Dr. E. S. Guzman, Department of Medicine, The University of Chicago, Chicago, Illinois.

Barth, Dr. L. G., Department of Zoology, Columbia University, New York City, New York.

Bartlett, Dr. James H., Department of Physics, University of Illinois, Urbana, Illinois.

Beadle, Dr. G. W., School of Biological Sciences, Stanford University, California.

Beams, Dr. Harold W., Department of Zoology, State University of Iowa, Iowa City, Iowa.

Beck, Dr. L. V., Hahnemann Medical College, Philadelphia, Pennsylvania.

Behre, Dr. Elinor H., Louisiana State University, Baton Rouge, Louisiana.

Bertholf, Dr. Lloyd M., Western Maryland College, Westminster, Maryland.

Bigelow, Dr. H. B., Museum of Comparative Zoology, Cambridge, Massachusetts.

Bigelow, Prof. R. P., Massachusetts Institute of Technology, Cambridge, Massachusetts.

Binford, Prof. Raymond, Guilford College, North Carolina.

Bissonnette, Dr. T. Hume, Trinity College, Hartford, Connecticut.

Blanchard, Prof. K. C., Johns Hopkins Medical School, Baltimore, Maryland.

Bodine, Dr. J. H., Department of Zoology, State University of Iowa, Iowa City, Iowa.

Boring, Dr. Alice M., Yenching University, Peking, China.

Bradley, Prof. Harold C., University of Wisconsin, Madison, Wisconsin.

Brodie, Mr. Donald M., 522 Fifth Avenue, New York City, New York.

Bronfenbrenner, Dr. Jacques J., Department of Bacteriology, Washington University Medical School, St. Louis, Missouri.

Brooks, Dr. Matilda M., University of California, Department of Zoology, Berkeley, California.

Brooks, Dr. S. C., University of California, Berkeley, California.

Brown, Dr. Dugald E. S., New York University, College of Dentistry, 209 East 23d Street, New York City, New York.

Brown, Dr. Frank A., Jr., Department of Zoology, Northwestern University, Evanston, Illinois.

Buckingham, Miss Edith N., Sudbury, Massachusetts.

Buck, Dr. John B., Department of Zoology, University of Rochester, Rochester, New York.

Budington, Prof. R. A., Winter Park, Florida.

Bullington, Dr. W. E., Randolph-Macon College, Ashland, Virginia.

Burbanck, Dr. William D., Department of Biology, Drury College, Springfield, Missouri.

Burkenroad, Dr. M. D., Yale University, New Haven, Connecticut.

Byrnes, Dr. Esther F., 1803 North Camac Street, Philadelphia, Pennsylvania.

Cannan, Prof. R. K., New York University College of Medicine, 477 First Avenue, New York City, New York.

Carlson, Prof. A. J., Department of Physiology, The University of Chicago, Chicago, Illinois.

Carothers, Dr. E. Eleanor, 134 Avenue C. East, Kingman, Kansas.

Carpenter, Dr. Russell L., Tufts College, Tufts College, Massachusetts.

Carroll, Prof. Mitchell, Franklin and Marshall College, Lancaster, Pennsylvania.

Carver, Prof. Gail L., Mercer University, Macon, Georgia.

Cattell, Dr. McKeen, Cornell University Medical College. 1300 York Avenue, New York City, New York.

Cattell, Mr. Ware, 3609 Military Road, N. W., Washington, D. C.

Chambers, Dr. Robert, Washington Square College, New York University, Washington Square, New York City, New York.

Chase, Dr. Aurin M., Princeton University, Princeton, New Jersey.

Cheney, Dr. Ralph H., Biology Department, Long Island University, Brooklyn, New York.

Chidester, Prof. F. E., Auburndale, Massachusetts.

Child, Prof. C. M., Jordan Hall, Stanford University, California.

Churney, Lt. Leon, 28th Alt. Tng. Unit, HAAF, Harlingen, Texas.

Claff, Mr. C. Lloyd, Department of Biology, Brown University, Providence, Rhode Island.

Clark, Prof. E. R., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.

Clark, Dr. Leonard B., Department of Biology, Union College, Schenectady, New York.

Clarke, Dr. G. L., Woods Hole Oceanographic Institution, Woods Hole, Massachusetts.

Cleland, Prof. Ralph E., Indiana University, Bloomington, Indiana.

Clowes, Dr. G. H. A., Eli Lilly and Company, Indianapolis, Indiana.

Coe, Prof. W. R., Yale University, New Haven, Connecticut.

Cohn, Dr. Edwin J., 183 Brattle Street, Cambridge, Massachusetts.

COLE, DR. ELBERT C., Department of Biology, Williams College, Williamstown, Massachusetts.

COLE, DR. KENNETH S., University of Chicago, Chicago, Illinois.

COLLETT, DR. MARY E., Western Reserve University, Cleveland, Ohio.

COLTON, PROF. H. S., Box 601, Flagstaff, Arizona.

COOPER, DR. KENNETH W., Department of Biology, Princeton University, Princeton, New Jersey.

COPELAND, PROF. MANTON, Bowdoin College, Brunswick, Maine.

COSTELLO, DR. DONALD P., Department of Zoology, University of North Carolina, Chapel Hill, North Carolina.

COSTELLO, DR. HELEN MILLER, Department of Zoology, University of North Carolina, Chapel Hill, North Carolina.

CRAMPTON, PROF. H. E., American Museum of Natural History, New York City, New York.

CRANE, JOHN O., Woods Hole, Massachusetts.

CRANE, MRS. W. MURRAY, Woods Hole, Massachusetts.

CROASDALE, HANNAH T., Dartmouth College, Hanover, New Hampshire.

CROWELL, DR. P. S., JR., Department of Zoology, Miami University, Oxford, Ohio.

CURTIS, DR. MAYNIE R., 377 Dexter Trail, Mason, Michigan.

CURTIS, PROF. W. C., University of Missouri, Columbia, Missouri.

DAN, DR. KATSUMA, Misaki Biological Station, Misaki, Japan.

DAVIS, DR. DONALD W., College of William and Mary, Williamsburg, Virginia.

DAWSON, DR. A. B., Harvard University, Cambridge, Massachusetts.

DAWSON, DR. J. A., The College of the City of New York, New York City, New York.

DEDERER, DR. PAULINE H., Connecticut College, New London, Connecticut.

DEMEREC, DR. M., Carnegie Institution of Washington, Cold Spring Harbor, Long Island, New York.

DILLER, DR. WILLIAM F., 1016 South 45th Street, Philadelphia, Pennsylvania.

DODDS, PROF. G. S., Medical School, University of West Virginia, Morgantown, West Virginia.

DOLLEY, PROF. WILLIAM L., University of Buffalo, Buffalo, New York.

DONALDSON, DR. JOHN C., University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania.

DUBOIS, DR. EUGENE F., Cornell University Medical College, 1300 York Avenue, New York City, New York.

DUGGAR, DR. BENJAMIN M., c/o Lederle Laboratories Inc., Pearl River, New York.

DUNGAY, DR. NEIL S., Carleton College, Northfield, Minnesota.

DURYEE, DR. WILLIAM R., Surgeon General's Office, Washington, D. C.

EDWARDS, DR. D. J., Cornell University Medical College, 1300 York Avenue, New York City, New York.

ELLIS, DR. F. W., Monson, Massachusetts.

EVANS, DR. TITUS C., College of Physicians and Surgeons, 630 West 168th Street, New York City, New York.

FAILLA, DR. G., College of Physicians and Surgeons, 630 West 168th Street, New York City, New York.

Fauré-Fremiet, Prof. Emmanuel, Collège de France, Paris, France.
Faust, Dr. Ernest C., Tulane University of Louisiana, New Orleans, Louisiana.
Ferguson, Dr. James K. W., Department of Pharmacology, University of Toronto, Ontario, Canada.
Figge, Dr. F. H. J., 4636 Schenley Road, Baltimore, Maryland.
Fischer, Dr. Ernst, Department of Physiology, Medical College of Virginia, Richmond, Virginia.
Fisher, Dr. Jeanne M., Department of Biochemistry, University of Toronto, Toronto, Canada.
Fisher, Dr. Kenneth C., Department of Biology, University of Toronto, Toronto, Canada.
Forbes, Dr. Alexander, Harvard University Medical School, Boston, Massachusetts.
Frisch, Dr. John A., Canisius College, Buffalo, New York.
Furth, Dr. Jacob, Cornell University Medical College, 1300 York Avenue, New York City, New York.
Gage, Prof. S. H., Lock Box 70, Interlaken, New York.
Galtsoff, Dr. Paul S., 420 Cumberland Avenue, Somerset, Chevy Chase, Maryland.
Garrey, Prof. W. E., Vanderbilt University Medical School, Nashville, Tennessee.
Gates, Dr. Reginald R., Woods Hole, Massachusetts.
Geiser, Dr. S. W., Southern Methodist University, Dallas, Texas.
Gerard, Prof. R. W., The University of Chicago, Chicago, Illinois.
Glaser, Prof. O. C., Amherst College, Amherst, Massachusetts.
Goldforb, Prof. A. J., College of the City of New York, Convent Avenue and 139th Street, New York City, New York.
Goodchild, Dr. Chauncey G., State Teachers College, Springfield, Missouri.
Goodrich, Prof. H. B., Wesleyan University, Middletown, Connecticut.
Gottschall, Dr. Gertrude Y., 1630 Rhode Island Avenue, N.W., Washington, D. C.
Graham, Dr. J. Y., Roberts, Wisconsin.
Grand, Constantine G., Biology Department, Washington Square College, New York University, Washington Square, New York City, New York.
Grave, Prof. B. H., DePauw University, Greencastle, Indiana.
Gray, Prof. Irving E., Duke University, Durham, North Carolina.
Gregory, Dr. Louise H., Barnard College, Columbia University, New York City, New York.
Gudernatsch, Dr. J. Frederick, New York University, 100 Washington Square, New York City, New York.
Guthrie, Dr. Mary J., University of Missouri, Columbia, Missouri.
Guyer, Prof. M. F., University of Wisconsin, Madison, Wisconsin.
Hague, Dr. Florence, Sweet Briar College, Sweet Briar, Virginia.
Hall, Prof. Frank G., Duke University, Durham, North Carolina.
Hamburger, Dr. Viktor, Department of Zoology, Washington University, St. Louis, Missouri.
Hance, Dr. Robert T., The Cincinnati Milling Machine Co., Cincinnati 9, Ohio.
Hargitt, Prof. George T., Department of Zoology, Duke University, Durham, North Carolina.

Harman, Dr. Mary T., Kansas State Agricultural College, Manhattan, Kansas.
Harnly, Dr. Morris H., Washington Square College, New York University, New York City, New York.
Harper, Prof. R. A., R. No. 5, Bedford, Virginia.
Harrison, Prof. Ross G., Yale University, New Haven, Connecticut.
Hartline, Dr. H. Keffer, University of Pennsylvania, Philadelphia, Pennsylvania.
Hartman, Dr. Frank A., Hamilton Hall, Ohio State University, Columbus, Ohio.
Harvey, Dr. E. Newton, Guyot Hall, Princeton University, Princeton, New Jersey.
Harvey, Dr. Ethel Browne, 48 Cleveland Lane, Princeton, New Jersey.
Hayden, Dr. Margaret A., Wellesley College, Wellesley, Massachusetts.
Hayes, Dr. Frederick R., Zoological Laboratory, Dalhousie University, Halifax, Nova Scotia.
Haywood, Dr. Charlotte, Mount Holyoke College, South Hadley, Massachusetts.
Hecht, Dr. Selig, Columbia University, New York City, New York.
Heilbrunn, Dr. L. V., Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania.
Hendee, Dr. Esther Crissey, Russell Sage College, Troy, New York.
Henshaw, Dr. Paul S., National Cancer Institute, Bethesda, Maryland.
Hess, Prof. Walter N., Hamilton College, Clinton, New York.
Hiatt, Dr. E. P., New York University, 100 Washington Square, New York City, New York.
Hibbard, Dr. Hope, Department of Zoology, Oberlin College, Oberlin, Ohio.
Hill, Dr. Samuel E., Department of Biology, Russell Sage College, Troy, New York.
Hinrichs, Dr. Marie, Department of Physiology and Health Education, Southern Illinois Normal University, Carbondale, Illinois.
Hisaw, Dr. F. L., Harvard University, Cambridge, Massachusetts.
Hoadley, Dr. Leigh, Harvard University, Cambridge, Massachusetts.
Höber, Dr. Rudolf, University of Pennsylvania, Philadelphia, Pennsylvania.
Hodge, Dr. Charles, IV, Temple University, Department of Zoology, Philadelphia, Pennsylvania.
Hogue, Dr. Mary J., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.
Hollaender, Dr. Alexander, c/o National Institute of Health, Laboratory of Industrial Hygiene, Bethesda, Maryland.
Hopkins, Dr. Dwight L., Mundelein College, 6363 Sheridan Road, Chicago, Illinois.
Hopkins, Dr. Hoyt S., New York University, College of Dentistry, New York City, New York.
Howland, Dr. Ruth B., Washington Square College, New York University, Washington Square East, New York City, New York.
Hoyt, Dr. William D., Washington and Lee University, Lexington, Virginia.
Hyman, Dr. Libbie H., American Museum of Natural History, New York City, New York.
Irving, Prof. Laurence, Swarthmore College, Swarthmore, Pennsylvania.
Iselin, Mr. Columbus O'D., Woods Hole, Massachusetts.

Jacobs, Prof. Merkel H., School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

Jenkins, Dr. George B., 30 Gallatin Street, N.W., Washington, D. C.

Jennings, Prof. H. S., Department of Zoology, University of California, Los Angeles, California.

Johlin, Dr. J. M., Vanderbilt University Medical School, Nashville, Tennessee.

Jones, Dr. E. Ruffin, Jr., College of William and Mary, Williamsburg, Virginia.

Kaufmann, Prof. B. P., Carnegie Institution, Cold Spring Harbor, Long Island, New York.

Kempton, Prof. Rudolf T., Vassar College, Poughkeepsie, New York.

Kidder, Dr. George W., Brown University, Providence, Rhode Island.

Kille, Dr. Frank R., Swarthmore College, Swarthmore, Pennsylvania.

Kindred, Dr. J. E., University of Virginia, Charlottesville, Virginia.

King, Dr. Helen D., Wistar Institute of Anatomy and Biology, 36th Street and Woodland Avenue, Philadelphia, Pennsylvania.

King, Dr. Robert L., State University of Iowa, Iowa City, Iowa.

Knowlton, Prof. F. P., Syracuse University, Syracuse, New York.

Kopac, Dr. M. J., Washington Square College, New York University, New York City, New York.

Korr, Dr. I. M., Department of Physiology, New York University, College of Medicine, 477 First Avenue, New York City, New York.

Krahl, Dr. M. E., College of Physicians and Surgeons, 630 West 168th Street, New York 32, New York.

Krieg, Dr. Wendell J. S., 303 East Chicago Ave., Chicago, Illinois.

Lancefield, Dr. D. E., Queens College, Flushing, New York.

Lancefield, Dr. Rebecca C., Rockefeller Institute, 66th Street and York Avenue, New York City, New York.

Lange, Dr. Mathilde M., Wheaton College, Norton, Massachusetts.

Lavin, Dr. George I., Rockefeller Institute, 66th Street and York Avenue, New York City, New York.

Lewis, Prof. I. F., University of Virginia, Charlottesville, Virginia.

Lillie, Prof. Frank R., The University of Chicago, Chicago, Illinois.

Lillie, Prof. Ralph S., The University of Chicago, Chicago, Illinois.

Little, Dr. E. P., Phillips Exeter Academy, Exeter, New Hampshire.

Lochhead, Dr. John H., Department of Zoology, University of Vermont, Burlington, Vermont.

Loeb, Prof. Leo, 40 Crestwood Drive, St. Louis, Missouri.

Loewi, Prof. Otto, 155 East 93d Street, New York City, New York.

Lowther, Mrs. Florence deL., Barnard College, Columbia University, New York City, New York.

Lucas, Dr. Alfred M., Regional Poultry Research Laboratory, East Lansing, Michigan.

Lucké, Prof. Balduin, University of Pennsylvania, Philadelphia, Pennsylvania.

Lynch, Dr. Clara J., Rockefeller Institute, 66th Street and York Avenue, New York City, New York.

Lynch, Dr. Ruth Stocking, Maryland State Teachers College, Towson, Maryland.

Lynn, Dr. William G., Department of Biology, The Catholic University of America, Washington, D. C.

MacDougall, Dr. Mary S., Agnes Scott College, Decatur, Georgia.

MacNaught, Mr. Frank M., Marine Biological Laboratory, Woods Hole, Massachusetts.

McClung, Prof. C. E., 417 Harvard Avenue, Swarthmore, Pennsylvania.

McCouch, Dr. Margaret Sumwalt, University of Pennsylvania Medical School, Philadelphia, Pa.

McGregor, Dr. J. H., Columbia University, New York City, New York.

Macklin, Dr. Charles C., School of Medicine, University of Western Ontario, London, Canada.

Magruder, Dr. Samuel R., Department of Anatomy, Tufts Medical School, Boston, Massachusetts.

Malone, Prof. E. F., College of Medicine, University of Cincinnati, Department of Anatomy, Cincinnati, Ohio.

Manwell, Dr. Reginald D., Syracuse University, Syracuse, New York.

Marsland, Dr. Douglas A., Washington Square College, New York University, New York City, New York.

Martin, Prof. E. A., Department of Biology, Brooklyn College, Bedford Avenue and Avenue H, Brooklyn, New York.

Mast, Prof. S. O., Johns Hopkins University, Baltimore, Maryland.

Mathews, Prof. A. P., Woods Hole, Massachusetts.

Matthews, Dr. Samuel A., Thompson Biological Laboratory, Williams College, Williamstown, Massachusetts.

Mavor, Prof. James W., Union College, Schenectady, New York.

Mazia, Dr. Daniel, Department of Zoology, Gowen Field, Boise, Idaho.

Medes, Dr. Grace, Lankenau Research Institute, Philadelphia, Pennsylvania.

Meigs, Mrs. E. B., 1736 M Street, N.W., Washington, D. C.

Menkin, Dr. Valy, Duke University, School of Medicine, Durham, North Carolina.

Metz, Prof. Charles W., University of Pennsylvania, Philadelphia, Pennsylvania.

Michaelis, Dr. Leonor, Rockefeller Institute, 66th Street and York Avenue, New York City, New York.

Miller, Dr. J. A., Division of Anatomy, College of Medicine, University of Tennessee, Memphis, Tennessee.

Minnich, Prof. D. E., Department of Zoology, University of Minnesota, Minneapolis, Minnesota.

Mitchell, Dr. Philip H., Brown University, Providence, Rhode Island.

Moore, Dr. Carl R., The University of Chicago, Chicago, Illinois.

Morgan, Dr. Isabel M., Poleomyelitis Research Center, 1901 E. Madison Street, Baltimore 5, Maryland.

Morgulis, Dr. Sergius, University of Nebraska, Omaha, Nebraska.

Morrill, Prof. C. V., Cornell University Medical College, 1300 York Avenue, New York City, New York.

Muller, Prof. H. J., Amherst College, Amherst, Massachusetts.

Nachmansohn, Dr. D., College of Physicians and Surgeons, 630 W. 168th Street, New York City, New York.

Navez, Dr. Albert E., Department of Biology, Milton Academy, Milton, Massachusetts.

Newman, Prof. H. H., 173 Devon Drive, Clearwater, Florida.

Nichols, Dr. M. Louise, Rosemont, Pennsylvania.

Nonidez, Dr. José F., Cornell University Medical College, 1300 York Avenue, New York City, New York.

Northrop, Dr. John H., The Rockefeller Institute, Princeton, New Jersey.

Ochoa, Dr. Severo, New York University, College of Medicine, 477 First Avenue, New York 16, New York.

Oppenheimer, Dr. Jane M., Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania.

Osburn, Prof. R. C., Ohio State University, Columbus, Ohio.

Osterhout, Prof. W. J. V., Rockefeller Institute, 66th Street and York Avenue, New York City, New York.

Osterhout, Mrs. Marian Irwin, Rockefeller Institute, 66th Street and York Avenue, New York City, New York.

Packard, Dr. Charles, Marine Biological Laboratory, Woods Hole, Massachusetts.

Page, Dr. Irvine H., Cleveland Clinic, Cleveland, Ohio.

Pappenheimer, Dr. A. M., Columbia University, New York City, New York.

Parker, Prof. G. H., Harvard University, Cambridge, Massachusetts.

Parmenter, Dr. C. L., Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania.

Parpart, Dr. Arthur K., Princeton University, Princeton, New Jersey.

Patten, Dr. Bradley M., University of Michigan Medical School, Ann Arbor, Michigan.

Payne, Prof. F., University of Indiana, Bloomington, Indiana.

Peebles, Prof. Florence, Lewis and Clark College, Portland, Oregon.

Pierce, Dr. Madelene E., Vassar College, Poughkeepsie, New York.

Pinney, Dr. Mary E., Milwaukee-Downer College, Milwaukee, Wisconsin.

Plough, Prof. Harold H., Amherst College, Amherst, Massachusetts.

Pollister, Dr. A. W., Columbia University, New York City, New York.

Pond, Dr. Samuel E., 1203 Enfield Street, Thompsonville, Connecticut.

Pratt, Dr. Frederick H., Boston University, School of Medicine, Boston, Massachusetts.

Prosser, Dr. C. Ladd, University of Chicago, Chicago, Illinois.

Rand, Dr. Herbert W., Harvard University, Cambridge, Massachusetts.

Rankin, Dr. John S., Zoology Department, University of Connecticut, Storrs, Connecticut.

Redfield, Dr. Alfred C., Harvard University, Cambridge, Massachusetts.

Renn, Dr. Charles E., Harvard University, Cambridge, Massachusetts.

Renshaw, Prof. Birdsey, Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York City, New York.

deRenyi, Dr. George S., Department of Anatomy, University of Pennsylvania, Philadelphia, Pennsylvania.

Reznikoff, Dr. Paul, Cornell University Medical College, 1300 York Avenue, New York City, New York.

Rice, Prof. Edward L., Ohio Wesleyan University, Delaware, Ohio.

Richards, Prof. A., University of Oklahoma, Norman, Oklahoma.

Richards, Prof. A. G., Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania.

Richards, Dr. O. W., Research Department, Spencer Lens Company, 19 Doat Street, Buffalo, New York.

Riggs, Lawrason, Jr., 120 Broadway, New York City, New York.

Rogers, Prof. Charles G., Oberlin College, Oberlin, Ohio.

Rogick, Dr. Mary D., College of New Rochelle, New Rochelle, New York.

Romer, Dr. Alfred S., Harvard University, Cambridge, Massachusetts.

Root, Dr. R. W., Department of Biology, College of the City of New York, Convent Avenue and 139th Street, New York City, New York.

Root, Dr. W. S., College of Physicians and Surgeons, Department of Physiology, 630 West 168th Street, New York City, New York.

Ruebush, Dr. T. K., Dayton, Virginia.

Rugh, Dr. Roberts, Department of Biology, Washington Square College, New York University, New York City, New York.

Saslow, Dr. George, Washington University Medical School, St. Louis, Missouri.

Saunders, Laurence, President, Saunders Publishing Company, Philadelphia, Pennsylvania.

Sayles, Dr. Leonard P., Department of Biology, College of the City of New York, 139th Street and Convent Avenue, New York City, New York.

Schaeffer, Dr. Asa A., Biology Department, Temple University, Philadelphia, Pennsylvania.

Scharrer, Dr. Ernst A., Western Reserve University, School of Medicine, 2109 Adelbert Road, Cleveland 6, Ohio.

Schechter, Dr. Victor, College of the City of New York, 139th Street and Convent Avenue, New York City, New York.

Schmidt, Dr. L. H., Christ Hospital, Cincinnati, Ohio.

Schmitt, Prof. F. O., Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Schotté, Dr. Oscar E., Department of Biology, Amherst College, Amherst, Massachusetts.

Schrader, Dr. Franz, Department of Zoology, Columbia University, New York City, New York.

Schrader, Dr. Sally Hughes, Department of Zoology, Columbia University, New York City, New York.

Schramm, Prof. J. R., University of Pennsylvania, Philadelphia, Pennsylvania.

Scott, Dr. Allan C., Union College, Schenectady, New York.

Scott, Prof. William B., 7 Cleveland Lane, Princeton, New Jersey.

Scott, Sister Florence Marie, Professor of Biology, Seton Hill College, Greensburg, Pennsylvania.

Semple, Mrs. R. Bowling, 140 Columbia Heights, Brooklyn, New York.

Severinghaus, Dr. Aura E., Department of Anatomy, College of Physicians and Surgeons, 630 West 168th Street, New York City, New York.

Shanes, Dr. Abraham M., New York University, College of Dentistry, New York.

Shapiro, Dr. Herbert, Radiation Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Shelford, Prof. V. E., Vivarium, Wright and Healey Streets, Champaign, Illinois.
Shull, Prof. A. Franklin, University of Michigan, Ann Arbor, Michigan.
Shumway, Dr. Waldo, University of Illinois, Urbana, Illinois.
Sichel, Dr. Ferdinand J. M., University of Vermont, Burlington, Vermont.
Sichel, Mrs. F. J. M., 35 Henderson Terrace, Burlington, Vermont.
Sinnott, Dr. E. W., Osborn Botanical Laboratory, Yale University, New Haven, Connecticut.
Slifer, Dr. Eleanor H., Department of Zoology, State University of Iowa, Iowa City, Iowa.
Smith, Dr. Dietrich Conrad, Department of Physiology, University of Maryland School of Medicine, Lombard and Greene Streets, Baltimore, Maryland.
Snyder, Prof. L. H., Ohio State University, Department of Zoology, Columbus, Ohio.
Sollman, Dr. Torald, Western Reserve University, Cleveland, Ohio.
Sonneborn, Dr. T. M., Department of Zoology, Indiana University, Bloomington, Indiana.
Speidel, Dr. Carl C., University of Virginia, University, Virginia.
Stark, Dr. Mary B., 1 East 105th Street, New York City, New York.
Steinbach, Dr. H. Burr, Department of Zoology, Washington University, St. Louis, Missouri.
Stern, Dr. Curt, Department of Zoology, University of Rochester, Rochester, New York.
Stern, Dr. Kurt G., Polytechnic Institute, Department of Chemistry, 85 Livingston Street, Brooklyn, New York.
Stewart, Dr. Dorothy R., University of Pennsylvania Medical School, Department of Physiology, Philadelphia 4, Pennsylvania.
Stokey, Dr. Alma G., Department of Botany, Mount Holyoke College, South Hadley, Massachusetts.
Strong, Prof. O. S., College of Physicians and Surgeons, Columbia University, New York City, New York.
Stunkard, Dr. Horace W., New York University, University Heights, New York.
Sturtevant, Dr. Alfred H., California Institute of Technology, Pasadena, California.
Summers, Dr. Francis Marion, R.F.D. Route 2, Box 507-A, Dinuba, California.
Taft, Dr. Charles H., Jr., University of Texas Medical School, Galveston, Texas.
Tashiro, Dr. Shiro, Medical College, University of Cincinnati, Cincinnati, Ohio.
Taylor, Dr. C. V., Leland Stanford University, Leland Stanford, California.
Taylor, Dr. William R., University of Michigan, Ann Arbor, Michigan.
TeWinkel, Dr. L. E., Department of Zoology, Smith College, Northampton, Massachusetts.
Turner, Dr. Abby H., Wilson College, Chambersburg, Pennsylvania.
Turner, Prof. C. L., Northwestern University, Evanston, Illinois.
Tyler, Dr. Albert, California Institute of Technology, Pasadena, California.
Uhlenhuth, Dr. Eduard, University of Maryland, School of Medicine, Baltimore, Maryland.
Visscher, Dr. J. Paul, Western Reserve University, Cleveland, Ohio.

WALD, DR. GEORGE, Biological Laboratories, Harvard University, Cambridge, Massachusetts.

WARBASSE, DR. JAMES P., Woods Hole, Massachusetts.

WARD, PROF. HENRY B., 1201 W. Nevada, Urbana, Illinois.

WARREN, DR. HERBERT S., 1405 Greywall Lane, Overbrook Hills, Pennsylvania.

WATERMAN, DR. ALLYN J., Department of Biology, Williams College, Williamstown, Massachusetts.

WEISS, DR. PAUL A., Department of Zoology, The University of Chicago, Chicago, Illinois.

WENRICH, DR. D. H., University of Pennsylvania, Philadelphia, Pennsylvania.

WHEDON, DR. A. D., North Dakota Agricultural College, Fargo, North Dakota.

WHITAKER, DR. DOUGLAS M., P. O. Box 2514, Stanford University, California.

WHITE, DR. E. GRACE, Wilson College, Chambersburg, Pennsylvania.

WHITING, DR. PHINEAS W., Zoological Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania.

WHITNEY, DR. DAVID D., University of Nebraska, Lincoln, Nebraska.

WICHTERMAN, DR. RALPH, Biology Department, Temple University, Philadelphia, Pennsylvania.

WIEMAN, PROF. H. L., University of Cincinnati, Cincinnati, Ohio.

WILLIER, DR. B. H., Department of Biology, Johns Hopkins University, Baltimore, Maryland.

WILSON, DR. J. W., Brown University, Providence, Rhode Island.

WITSCHI, PROF. EMIL, Department of Zoology, State University of Iowa, Iowa City, Iowa.

WOLF, DR. ERNST, Biological Laboratories, Harvard University, Cambridge, Massachusetts.

WOODRUFF, PROF. L. L., Yale University, New Haven, Connecticut.

WOODWARD, DR. ALVALYN E., Zoology Department, University of Michigan, Ann Arbor, Michigan.

WRINCH, DR. DOROTHY, Smith College, Northampton, Massachusetts.

YNTEMA, DR. C. L., Department of Anatomy, Cornell University Medical College, 1300 York Avenue, New York City, New York.

YOUNG, DR. B. P., Cornell University, Ithaca, New York.

YOUNG, DR. D. B., 7128 Hampden Lane, Bethesda, Maryland.

DOMINANT LETHALITY AND CORRELATED CHROMOSOME EFFECTS IN HABROBRACON EGGS X-RAYED IN DIPLOTENE AND IN LATE METAPHASE I[1]

ANNA R. WHITING

University of Pennsylvania

Introduction

If oviposition is prevented in well-fed females of the parasitic wasp Habrobracon by witholding them from their host they continue to produce mature eggs until the egg sacs are filled. These stored eggs may number as many as twenty per female and are in late metaphase of the first meiotic division (metaphase I). Their retention in this stage for four days has no effect on their hatchability which is 96 per cent in the wild type stock used for the experiments herein described.

When unmated females with stored eggs are x-rayed and allowed to oviposit at 30° C. all eggs laid during the first six hours after treatment will have been irradiated in late metaphase I. Eggs laid during the seventh and eighth hours after treatment consist of a variable mixture treated in metaphase I and in late diplotene (including all eggs in diakinesis) and are, therefore, of no use in the present study. Eggs laid during the ninth to twelfth hours after treatment will have been post-synaptic with their diffuse chromosomes in a relatively quiescent condition when irradiated. These are designated as late and early diplotene.

An advantage in the use of these eggs for the detection of injuries lies in the fact that they develop parthenogenetically if unfertilized and so indicate directly the effects of treatment on a haploid set of chromosomes. Disadvantages are the large number ($n = 10$) and small size (less than 1 μ in diameter) of their chromosomes. The details of oogenesis appear to be orthodox and so the results should be universally applicable to forms with comparable type of meiosis. Failure to hatch and cytological changes in stages immediately following treatment have been the criteria of injury. Preliminary results were first published in 1938 (Whiting, 1938). Details of technique and hatchability effects, as well as extensive bibliography, are given elsewhere (Whiting, 1945); cytological effects and their correlation with mortality and dose are presented here in detail.

Dose-hatchability Relationships

Hatchability effects may be summarized briefly. No correction for control hatchability is made since it is so close to 100 per cent. Eggs x-rayed in diplotene and allowed to develop parthenogenetically give a dose-hatchability curve which appears to be linear at low doses and to become "mixed" at high doses; they have 50 per cent mortality at 12,000 *r* and 100 per cent at about 45,000 *r*; they showed no significant change in hatchability in preliminary and inadequate tests of time-intensity differences. Those treated in early diplotene (laid during the eleventh and twelfth hours after treatment) show no change in hatchability at any dose with fractionated treatment, those treated in late diplotene (laid during the ninth and tenth hours after treatment) show a significant increase at high doses with fractionated treatment. The dose-hatchability curve for combined diplotene owes much of its mixed character at high doses to late diplotene, early diplotene response being more nearly linear.

---

[1] This investigation has been aided by a grant (to P. W. Whiting) from the Rockefeller Foundation, for apparatus and technical assistance. The work was done at the Zoological Laboratory of the University of Pennsylvania and at the Marine Biological Laboratory, Woods Hole, Massachusetts. To these institutions the author is grateful for the use of laboratory facilities and of x-ray equipment. Valuable assistance was also given by the American Oncologic Hospital of Philadelphia through the use of x-ray equipment.

Eggs x-rayed in late metaphase I and allowed to develop parthenogenetically show a linear decline in hatchability with increasing dose and have 50 per cent mortality at 375 *r*, 100 per cent at about 1,400 *r*; they show no change in dose-hatchability relationships with aging between treatment and oviposition, time-intensity differences or fractionation of dose.

When Habrobracon females are mated, about two-thirds of the eggs are fertilized. If treated females are mated to untreated males, the survival of any appreciable number of eggs through the aid of normal spermatozoa would increase percentage of hatchability thereby indicating the presence of recessive lethal effects by comparison with hatchability of eggs from treated unmated females. Table I demonstrates that most, if not all, of the lethal effects induced in these stages by x-rays are dominant, at least in respect to hatchability. This is rather surprising at first glance but in treated metaphase I, as pointed out below, chromosomal deletions appear to be relatively large and in either stage, it is possible that deletions small enough to act as recessives in fertilized eggs may not kill the individual until after hatching in unfertilized eggs. Lethals which are recessive in diploids may be due to such minute losses as to exert their effects only after hatching in haploids. Perhaps viable deficiency heterozygotes are so rare that hatchability of irradiated eggs is not perceptibly altered by fertilization with untreated spermatozoa. In any case, conditions are well suited to an analysis of dominant lethal ratios induced by x-rays in identifiable stages of meiosis and, although the chromosomes present

TABLE I

Hatchability percentages for eggs of treated females, unmated and mated to untreated males

Stage treated	Dose in *r* units	Unmated females		Mated females	
		Number of eggs	Hatchability percentage	Number of eggs	Hatchability percentage
Metaphase I	560	319	39.8±2.7	318	40.5±2.7
Prophase I	5,600	137	71.5±2.6	126	70.6±4.0
	22,400	100	19.0±3.9	182	19.2±2.9
Controls	0	127	98.4±1.1	363	98.6±0.6

difficulties, the eggs themselves are easily handled, fixed and stained for observation.

About 40,000 eggs were collected and observed for hatchability. Records were kept of the results from individual females in all cases so that aberrant behavior in eggs from any individual could be recognized. Such behavior was extremely rare.

From the work of Sax (1938, 1940), Fabergé (1940) and others on dose-chromosome injury curves, certain tentative conclusions were drawn concerning cytological effects before study of chromosomes was begun. For diplotene it was assumed that the great majority of chromosome breaks must undergo restitution; that broken ends of chromosomes within the same cell increase as dose increases, permitting complicated reunions (translocations, large interstitial deletions) so that lethal individual chromosome changes tend to be due increasingly to more than one ionization, especially in late diplotene; that bridges can be formed in either meiotic division or in both, due to lateral fusion of the broken ends of chromatids whenever two adjoining chromatids are broken by a single ionization. From the work of Sturtevant and Beadle (1936) and of McClintock (1941) it was thought that bridges in division I might be permanent or delayed in breakage and might offer an explanation for some, at least, of the high resistance of this stage to irradiation.

Concerning metaphase I it was assumed, because of the linear relationship of hatchability to dose, that injuries were in the form of terminal deletions or of minute interstitial deletions, in other words, injuries due to single ionizations. The high sensitivity of this stage suggested that most injuries must be permanent. It was doubted that a single ionization would break two chromatids due to the degree of separation in late metaphase I and so the occurrence of bridges in either division from this cause seemed improbable. There was also the possibility that high metaphase sensitivity might be due to "physiological" effects, stickiness, etc., which would result in fusion bridges, delay in division or death.

Cytological Observations

Cytology of controls. The cytology of the stages before metaphase I has not been studied in detail, either in control or irradiated material, because of the small size and large numbers of chromosomes and of their elongate and diffuse condition. Synapsis occurs in very young oocytes and the subsequent behavior through condensation appears to have nothing exceptional about it. Changes take place slowly and are not obvious in character until just before condensation of chromosomes (diakinesis) when tetrads move to periphery of the nucleus. Most students of hymenopteran cytology would question the conclusion that the stored oocyte is in an orthodox and identifiable stage, late metaphase I. They state that the chromatin has reverted to a resting stage or has formed an abortive spindle, a compact clump or a composite body, etc. Speicher (1936) finds that the most advanced eggs in the Habrobracon egg sac are in "early anaphase of the first maturation" which the author prefers to call late metaphase. Speicher's observations that distinct chromosomes are present, are in the form of tetrads (Fig. 1) and are ten in number has been repeatedly checked by the author and cannot be questioned. They show the forms expected for tetrads and each resolves immediately into two pairs of dyads upon completing division I. The conclusion must be drawn either that Habrobracon differs from many other Hymenoptera in having orthodox oogenesis or that its chromosomes retain more easily their individuality when fixed.

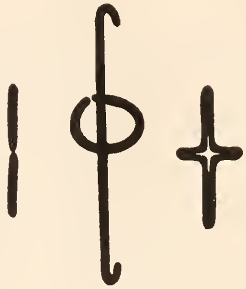


FIGURE 1. Three tetrads from one late metaphase I spindle. Untreated. Drawn from whole mount of egg with aid of a camera lucida. Semi-diagrammatic. × 4,625.

The stages of normal oogenesis following oviposition, as described by Speicher, are briefly as follows. During the process of oviposition the maturation spindle is moved from dorsal to ventral side of the egg. It then passes into telophase I. The second division follows immediately. The four groups of chromosomes (1a, 1b, 2a, 2b) lie in a row roughly perpendicular to the egg surface. During anaphase II polar nuclei 1a and 2a remain stationary, 1b moves close to 2a, and 2b (functional nucleus) sinks deeper into the egg, a membrane forming as it moves. Nucleus 1a soon disintegrates, 1b and 2a unite and form a metaphase plate which divides and then disintegrates. Cleavage is of the usual insect type, with nuclei moving about until blastoderm formation when cell membranes first appear. The stages following oviposition are the ones which were studied after irradiation.

No evidences of displaced chromosomes or of aberrant conditions resembling those observed in irradiated eggs were found by Speicher or by the author in large numbers of control eggs studied.

Cytology of irradiated eggs. In experiments concerned with cytological effects, eggs from control and treated females were incubated according to standard schedules, dropped into fixative (formalin-acetic-alcohol), punctured at the posterior end to facilitate fixation, treated with the Feulgen technique and mounted whole in balsam. Control hatchability tests were made of eggs treated at the same time as those fixed. Slides were made of about 2,500 eggs.

After treatment in diplotene acentric fragments, dicentrics or both may occur in division I (Fig. 2a, b, c) or in division II or in both divisions. Bridges in division I may be permanent and can be seen bulging at the side when nucleus 1b moves towards 2a, indicating that chromatin bridges are tensile but not elastic (Fig. 2c). Acentric fragments remain visible throughout both divisions. No evidences of stickiness or of clumping of chromatin (Fig. 2a, b, c) or of retardation of meiosis are apparent for doses up to lethal (45,000 r). Of eggs treated in diplotene with 44,800 r, 1.1 per cent died at first cleavage, 30.4 per cent with a few nuclei, 54.3 per cent with many nuclei and 14.2 per cent at blastoderm.

Immediately after irradiation in late metaphase I (Fig. 1), chromosomes show no apparent change but at telophase I acentric fragments are left within the spindle and these remain visible throughout division II (Fig. 2d, e). They are often almost as large as entire chromosomes and can usually be identified as double structures. No bridges have been seen in division I in over 1,500 eggs observed. In division II bridges occur and after heavy treatment (2,000 *r*) several may be seen in each second division spindle (Fig. 2e). Small fragments occasionally appear in division II spindles. No evidences of stickiness or of clumping (Fig. 2d, e) or of retardation of meiosis occur in development following treatment with lethal dose of this stage which, except for absence of bridges in division I, behaves cytologically as treated diplotene. Percentages of eggs with fragments in division I and mean number of fragments per treated increase linearly with increased dose (Whiting, 1945). All eggs exposed in late metaphase I to 2,016 *r* undergo some development. 2.4 per cent die in first cleavage, 7.2 per cent with a few nuclei, 71.4 per cent with many nuclei and 19.0 per cent at blastoderm stage. In spite of their high sensitivity, some eggs treated in metaphase I developed to the fifth cleavage (expected) after 15,000 *r*, to metaphase II after 25,000 *r*, to pronucleus after 35,000 *r* and one to anaphase II after 200,000 *r*. No records were kept of rate of development at these higher doses.

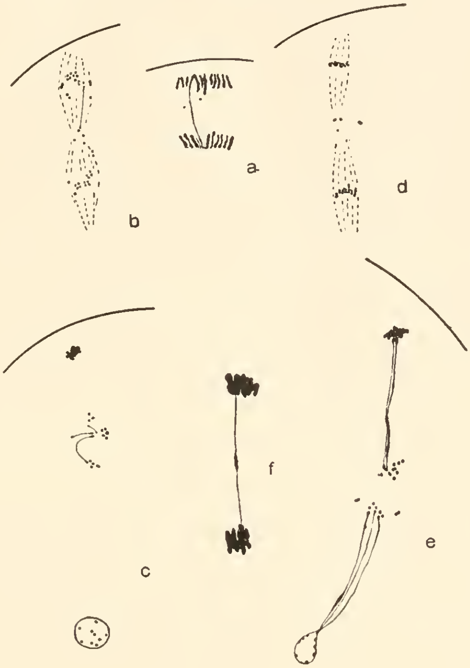


FIGURE 2. Illustrations were drawn from whole mounts of eggs with the aid of a camera lucida. *a, b,* and *c,* eggs were irradiated in late diplotene with 44,800 *r*; *a,* telophase I. × 1,500, *b,* metaphase II. × 875; *c,* telophase II. × 1,250; *d, e,* and *f,* eggs were irradiated in late metaphase I with 2,000 *r*; *d,* metaphase II. × 875; *e,* telophase II. × 1,250; *f,* third cleavage, telophase. × 4,550.

The similar patterns of stage at death for both diplotene and metaphase I at their respective lethal doses indicate that, in spite of the great difference in sensitivity between the stages, cause of death is of the same nature in both. These data on time of death check what has often been noted, especially in respect to mature spermatozoa, that so-called lethal doses are not actually lethal to the treated cell itself but, instead, to its descendents. The fact that the oocyte continues to function normally and that death does not occur until it becomes an embryo, supports the argument that cytoplasmic injury is not at the basis of mortality. It is due, rather, to loss of parts of chromosomes during meiosis following irradiation and to resulting incomplete chromosome complements in every cleavage nucleus.

Bridges occur in cleavage I after treatment in either stage indicating that, if chromatids are already split when treated, there occurs lateral fusion of broken ends of half-chromatids. If they are not split when treated the split occurring in the first mitosis must have been incomplete in the broken chromatid or have resulted in lateral fusion of broken ends of daughter chromosomes. Bridges appear in subsequent cleavages. Fragments, which also occur in cleavage, could not be explained at first until it was noted that they are tapering at the ends and that they result from double breaks in a bridge which releases a thickened middle portion (Fig. 2f). Fragments were not observed after every mitosis in the same embryo although bridges, if present, appear in all cleavage figures.

Correlation of Injury with Chromosome Form when Treated

It is perhaps unwise to devote much time and space to the subject of the correlation of the nature of the injuries and the form of the chromosomes when treated in view of the small size of Habrobracon chromosomes and the disagreement of investigators in this field. Obviously, there is a correlation. The studies of Sax (1938, 1940), Fabergé (1940) and McClintock (1938) will be used as a basis of a brief discussion, since the results of these investigations are consistent with their theories.

Chromosome injuries fall into two classes, those caused by single ionizations and those caused by more than one. The former consist of terminal deletions and minute interstitial deletions. Two identical terminal deletions can be induced by a single ionization if two chromatids are sufficiently close together. When this happens, lateral fusion of broken ends occurs resulting in a dicentric, from parts of the two chromatids still attached to spindle fibers, and an acentric, from the released and fused ends. Single terminal deletions can be induced by single ionizations and this appears to be the rule when chromatids are widely separated. An acentric is ultimately lost and a dicentric forms a bridge when its two spindle fiber attachment points (centromeres) are pulled apart. If the bridge does not break, an entire chromosome may be missing from a daughter cell. If it does break, the resulting chromosomes are incomplete and each daughter cell will have an incomplete chromosome and, therefore, an incomplete set of genes. Such a terminally incomplete chromosome may continue to form a bridge in each subsequent division, either by failing to split completely or by a lateral fusion of the broken ends after splitting. This appears to be the general rule but McClintock (1941) has found that when such an incomplete chromosome occurs in the sporophyte tissue of maize, it forms no bridge.

An interstitial deletion caused by a single ionization in a chromosome would mean the loss of genes and would be lethal if they were numerous or of sufficient importance but it would not be cytologically apparent in subsequent divisions because of its small size.

Injuries which must be due to more than one ionization since they involve breaks in chromosomes too far apart to be caused by a single ionization are large inversions, large interstitial deletions and translocations. Inversions would not be apparent, either cytologically or in effect on viability of the embryo receiving them in the present study since they would be induced after synapsis and crossing over and the inversion of a block of genes would probably have no lethal effect. Large interstitial deletions would have a lethal effect but could not be identified in material used in these experiments. Translocations might be lethal and would be visible as bridges should centric parts of non-homologues become attached to each other. Such bridges cannot be distinguished cytologically from those resulting from double terminal deletions in this material.

The nature of the hatchability curves suggests that most injuries in early diplotene and late metaphase I at all doses and in late diplotene at low doses are caused by single ionizations, that many injuries in late diplotene at high doses are caused by more than one ionization. Since there is no reason to suppose that the nature of original breaks would be changed by higher doses it is presumed that the number of single breaks per cell increases with high dose and allows greater opportunity for new combinations because of increased number of broken ends available at any one time. This would take for granted the breaking of single chromatids per ionization for if two were broken the lateral fusion of broken ends would prevent translocations, fusion with more distant chromosomes. The reduction in injury by fractionation of dose is explained on the grounds that, with repeated smaller doses, fewer free ends are available at any given time for new combinations and the intervals between treatments afford an opportunity for restitution or changes in broken ends to occur so that they are no longer capable of joining with other broken ends formed by later treatments.

Three conditions seem to be of importance, then, in determining response of the chromosomes here studied to irradiation. These are (1) relation of tetrads to each other in the nucleus, (2) degree of separation of adjoining chromatids within a tetrad and (3) nature and degree of tension on chromosomes. Each of the three stages will be discussed briefly from these points of view.

In early diplotene the tetrads are evenly distributed within the nucleus, sister chromatids are in contact, homologues separated except at chiasmata, and neither traction of the spindle fibers nor terminalization has begun. Most breaks will be temporary because of lack of tension and relaxed state of the chromosomes. Translocations should be possible but the majority of breaks will involve both sister chromatids with the production of acentrics and dicentrics. Permanent double breaks can occur either between centromeres and proximal chiasmata (with production of bridge in division II) or distal to chiasmata (producing bridge in division I if distal to "odd" chiasmata, in division II if distal to "even") since the slight tension which exists is equally exerted everywhere along the length of the chromosome.

In late diplotene the tetrads move peripherally but are still widely separated, terminalization (movement of chiasmata towards ends of tetrads) has begun, as well as movement of centromeres away from each other, and chromatids are not so closely associated, especially toward ends of chromosomes. Single and double breaks will occur (the latter nearer the centromere) and more of them will be permanent because of new tensions. Bridges should be less frequent in division I than in the case of early diplotene but this has not been checked. This stage will be somewhat more sensitive and will exert its lethal effects through translocations and large interstitial deletions as well as through double terminal deletions.

In late metaphase I, the tetrads are isolated from each other and stable in position on the spindle so that interchanges between them would not be expected. Centromeres are pulled far from each other and chiasmata resist further terminalization (Fig. 1) so that tension exerted between centromeres and proximal chiasmata is very great, tension exerted distal to chiasmata not so great. Ionizations will cause double breaks near centromeres where sister chromatids are closely approximated and these will all be permanent because of the extreme tension. They will result in large double fragments (acentrics) in division I, bridges (dicentrics) in division II. Breaks induced towards ends of chromosomes, and especially distal to chiasmata, will be less likely to be permanent and more likely to be single. There will be few or no bridges in division I and single fragments will appear in division I or division II (McClintock, 1938).

Any injury to a tetrad which results in a single bridge in division II reduces the chance of hatching of the egg by fifty per cent; in division I the effect is the same if the bridge breaks promptly. If it is delayed in breaking or does not break the hatchability of the egg is not affected, since an incomplete chromatid is thereby restrained from entering the ootid nucleus. A single terminal deletion reduces the chance of hatching by twenty-five per cent.

With ten tetrads of the diverse forms found in Habrobracon, combinations of changes induced by single ionizations can become very complex. If added to these are the complication of translocation and of large interstitial deletions (characteristic especially, perhaps, of late diplotene) the great resistance of diplotene is to be wondered at. The author (Whiting, 1945) has reviewed the data here reported in the light of the numerous theories devised to explain differential sensitivity of chromosomes to x-rays and has found that the only one which applies is that put forth by Goodspeed in 1929. He suggested tension as the important factor. It seems highly probable that numerous breaks do occur in the evenly distributed, diffuse, slowly moving chromosomes of diplotene but that the majority of them is temporary. Healing or restitution must take place quickly for there is always some movement and these chromosomes ultimately go through the same stresses as those treated in late metaphase I and, in addition, those attendent upon condensation and complete terminalization. Their response to fractionation also argues for relatively rapid restitution.

The development of individuals, normal in appearance and in reproductive activity and with normal descendents, after treatment in diplotene with 35,000 *r*, illustrates graphically the resistance of this stage to permanent injury by ionizations.

Sax (1942) summarizes the information available on "physiological" effects of x-rays, one of which is stickiness of chromatin. It has been found that condensed chromosomes are most sensitive in respect to stickiness, that such effects are temporary, delay subsequent division, have a threshold dose, are lethal only after very high doses and result in "fusion" bridges if the cell divides before recovery. The stage in the present study most likely to show the effects of stickiness in the form of fusion bridges is division I after treatment of metaphase I. This is the only division which shows bridges of no kind even after doses much higher than lethal. A delay of twenty-four hours between treatment and resumption of meiosis does not increase hatchability, meiosis is not appreciably delayed after irradiation, there is no threshold effect (down to 50 *r*). It should be emphasized again that, wide apart as are the lethal doses for diplotene and metaphase I, at their respective lethal doses, the pattern of stages at death is the same, the same percentage dies at first cleavage, at blastoderm, etc.; in other words, 45,000 *r* has no more drastic effect on development of treated diplotene than 1,400 *r* on metaphase I. All evidence indicates that cause of death is of the same order for both stages and that "physiological" effects are of every minor importance, and not appreciably different in the two stages.

Sturtevant and Beadle (1936) failed to recover an expected genetic type of chromosome aberration correlated with a dicentric in division I. They suggested that in a form like Drosophila where the four meiotic nuclei lie in a row and where a terminal one alone functions, a bridge in division I might fail to break, or might be delayed in breaking, thereby tying together injured chromatids and allowing uninjured ones to pass to the terminal nuclei. McClintock (1941) also offers as explanation for the failure to obtain expected genetic results correlated with bridges in division I of maize, the selective effect of these bridges on broken chromatids. Terminal nuclei (one of which becomes the functional megaspore) tend to receive the uninjured chromatids. Figure 3c demonstrates that bridges in division I in Habrobracon eggs do not break, at least in some cases.

In divisions following treatment of diplotene to which this selection of injured chromatids for elimination would apply, the chances of having bridges in the second division are as frequent as in the first or more so and selection through permanence of bridges would apply, therefore, only in the simplest kind of injury and that to but one or very few tetrads since any number of breaks would be certain to produce some bridges in division. This selection, although it undoubtedly occurs, cannot explain more than a small amount of resistance of diplotene. It would be expected to apply especially with low doses when but a single break occurs in a single tetrad.

The wide difference in size of lethal doses (45,000 *r*–1,400 *r*) of such closely related stages of the same cell, the unreduced Habrobracon egg, confirms the truth of the conclusion made long ago (1906) by Krause and Ziegler in an extensive and critical study of tissue injury by x-rays, that it is less the kind of cell than its stage at the time of treatment which determines sensitivity.

The facts and theories just presented are of interest in connection with a discussion of dominant lethals by Pontecorvo (1942). He explains dominant lethal effects in Drosophila spermatozoa by assuming that single chromosome breaks are produced by radiations at a rate proportional to radiation dose and that these neither undergo restitution nor participate with other breaks in the same nucleus in rearrangements. "Chromosomes with broken ends give rise to a cycle of breakage-fusion-bridge phenomena in development." He also writes, "It is therefore an open question whether sister unions are so frequent as to cause a considerable portion of dominant lethality. Should this be the case, the trend of the curve of dominant lethality could be explained. Most dominant lethality would be determined by single-break sister unions at low dosages and as the dosage increased lethal changes of the other two types (translocations and deletions) would come to play an increasing part." Translocations and deletions would not be produced actually until syngamy since breaks appear to remain open in the sperm chromosomes until that time.

Broken chromosome ends do undergo restitution or participate with other broken ends in the egg very soon after treatment but the final contribution to the zygote may be the same as that made by the irradiated sperm; viz., a chromosome with a broken end which will give rise to the breakage-fusion-bridge cycle in the first cleavage as well as in subsequent ones. Most dominant lethality in the present study is, without much doubt, caused by single ionizations and only in late diplotene at high doses does treatment appear to cause a high percentage of death from the cooperation of two or more ionizations.

It is of interest in this connection to note that the dose-injury curve for dominant lethality in the spermatozoa of Drosophila (Sonnenblick, 1940; Demerec and Fano, 1944) and for Habrobracon (Heidenthal, 1945) is of the same nature as that for late diplotene.

CONCLUSION

1. (Tentative) The majority of dominant lethals induced in late diplotene by low doses (to 11,000 *r*) and in early diplotene and in late metaphase I by all doses through lethal, in Habrobracon eggs, is caused by single ionizations which break adjoining chromatids. Lateral fusion of broken ends results, followed by continued breakage-fusion-bridge phenomena in cleavage. With doses above 11,000 *r* in late diplotene an increasing number of lethal changes arises from two or more ionizations (translocations, large interstitial deletions). 2. Lethal doses are not lethal to the treated cell (oocyte) itself but to its descendents (embryo). Fragmentation of chromosomes is not lethal, loss of fragments is. 3. The nature and degree of chromosome injury can be correlated with the form of the chromosome and with forces acting upon it during and immediately following treatment. 4. The kind of cell is less important than its stage in determining sensitivity to x-rays. 5. Tension is the main factor in determining permanence of breaks caused by ionizations, chromosome form and movement in determining the nature of the new combinations of broken ends. 6. The chromosome phenomena here dealt with are common ones in the majority of animals and plants and it is predicted that, when metaphase and anaphase are sufficiently studied in other forms, they will be found to be the stages most sensitive to x-rays. This has proved to be the case for Sciara (Metz and Bozeman, 1940; Reynolds, 1941) and for Trillium (Sparrow, 1944).

SUMMARY

Unlaid Habrobracon eggs x-rayed in diplotene (lethal dose about 45,000 *r*) and allowed to develop parthenogenetically, show fragments, bridges or both in division I; either or both in division II. Bridges in division I may be permanent.

Unlaid eggs x-rayed in late metaphase I (lethal dose about 1,400 *r*) show fragments but no bridges in division I; bridges, fragments or both in division II.

An explanation of difference in cytological effects of x-rays on these stages and of the differences between them in sensitivity and in nature of survival curves is attempted through comparison with studies on forms with larger chromosomes.

LITERATURE CITED

Demerec, M., and U. Fano, 1944. Frequency of dominant lethals induced by radiation in sperms of Drosophila melanogaster. *Genetics*, **29**: 348–360.

Fabergé, A. C., 1940. An experiment on chromosome fragmentation in Tradescantia by X-rays. *Jour. Genetics*, **39**: 229–248.

Goodspeed, T. H., 1929. The effects of X-rays and radium on species of the genus Nicotiana. *Jour. Hered.*, **20**: 245–259.

Heidenthal, Gertrude, 1945. The occurrence of X-ray induced dominant lethal mutations in Habrobracon. *Genetics*, **30**: 197–205.

Krause, P., and K. Ziegler, 1906. Experimentelle Untersuchungen über die Einwirkung der Röntgenstrahlen auf tierisches Gewebe. *Fortschr. a. d. Geb. d. Röntgenstr.*, **10**: 126–182.

McClintock, Barbara, 1938. The fusion of broken ends of sister half-chromatids following chromatid breakage at meiotic anaphases. *Missouri Agric. Exp. Sta. Bull.*, **290**: 1–48.

McClintock, Barbara, 1941. The stability of broken ends of chromosomes in Zea mays. *Genetics*, **26**: 234–282.

Metz, C. W., and M. L. Bozeman, 1940. Further observations on the mechanism of induced chromosome rearrangements in Sciara. *Proc. Nat. Acad. Sci.*, **26**: 228–231.

Pontecorvo, G., 1942. The problem of dominant lethals. *Jour. Genetics*, **43**: 295–300.

Reynolds, J. P., 1941. X-ray induced chromosome rearrangements in the females of Sciara. *Proc. Nat. Acad. Sci.*, **27**: 204–208.

Sax, Karl, 1938. Chromosome aberrations induced by X-rays. *Genetics*, **23**: 494–516.

Sax, Karl, 1940. An analysis of X-ray induced chromosomal aberrations in Tradescantia. *Genetics*, **25**: 41–68.

Sax, Karl, 1942. The mechanism of X-ray effects on cells. *Jour. Gen. Physiol.*, **25**: 533–537.

Sonnenblick, B. P., 1940. Cytology and development of the embryos of X-rayed adult Drosophila melanogaster. *Proc. Nat. Acad. Sci.*, **26**: 373–381.

Sparrow, A. H., 1944. X-ray sensitivity changes in meiotic chromosomes and the nucleic acid cycle. *Proc. Nat. Acad. Sci.*, **30**: 147–155.

Speicher, B. R., 1936. Oogenesis, fertilization and early cleavage in Habrobracon. *Jour. Morph.*, **59**: 401–421.

Sturtevant, A. H., and G. W. Beadle, 1936. The relations of inversions in the X chromosome of Drosophila melanogaster to crossing over and disjunction. *Genetics*, **21**: 554–604.

Whiting, Anna R., 1938. Sensitivity to X-rays of stages in oogenesis of Habrobracon. *Rec. Genetics Soc. Am.*, **7**: 89.

Whiting, Anna R., 1945. Effects of X-rays on hatchability and on chromosomes of Habrobracon eggs treated in first meiotic prophase and metaphase. Amer. Naturalist, **79**: 193–227.

STRATIFICATION AND BREAKING OF THE ARBACIA PUNCTULATA EGG WHEN CENTRIFUGED IN SINGLE SALT SOLUTIONS

ETHEL BROWNE HARVEY

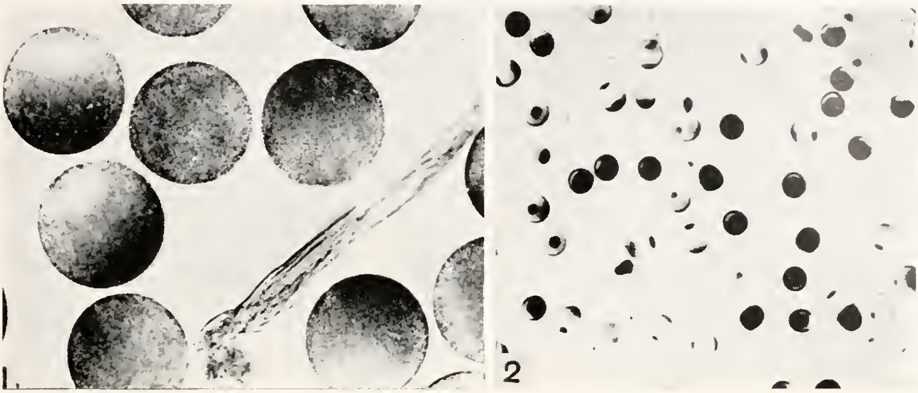
Marine Biological Laboratory, Woods Hole, and the Biological Laboratory, Princeton University

A study has been made of the comparative rate of stratification and breaking of the *Arbacia* egg in single salt solutions, when subjected to centrifugal force. It might be expected that when more rapid stratification occurs, the eggs would break apart more readily. This was, however, found not to be the case when the eggs were centrifuged in hypo- and hypertonic sea water, but this is probably due to the change in volume of the eggs (E. B. Harvey, 1943). With the increased surface area of the eggs in hypotonic sea water the tension at the surface is increased (Cole, 1932) and the eggs are more difficult to break apart. In the present experiments with pure salt solutions the surface area remained constant.

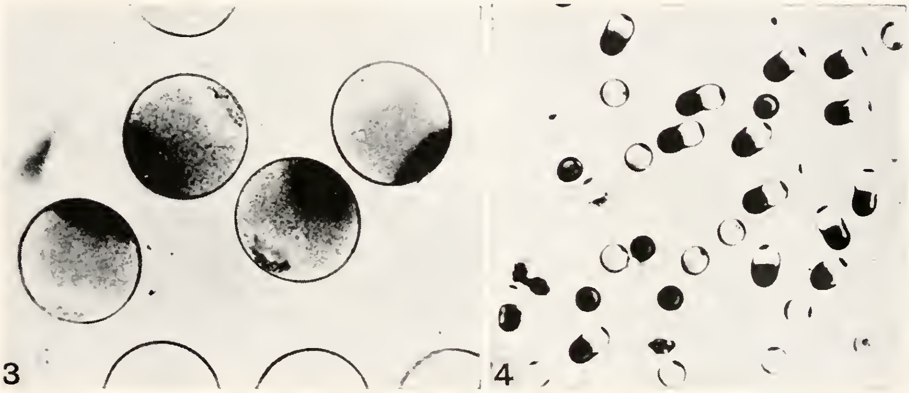
The solutions used in the following experiments were those routinely used at Woods Hole as isotonic with the sea water there, and found by me to be isosmotic on measuring the eggs after immersion, namely: 0.52 m NaCl, 0.53 m KCl, 0.34 m $CaCl_2$, and 0.37 m $MgCl_2$. The pH of the solutions was found to be respectively, 5.54, 5.44, 5.53, and 6.31. It was determined, however, that the pH in itself, at least of sea water, has no effect on the stratification and rate of breaking. Sea water was made up of pH ranging from 5 to 9 by adding HCl or NaOH; eggs kept in these solutions and centrifuged in them at the same time as those in normal sea water showed no difference in stratification or breaking. This was found also by Barth (1929) for stratification in sea water, though he did find an effect in NaCl. However Heilbrunn (1928, 1943) finds that Na definitely increases viscosity. The eggs were not injured by the pure salt solutions as they could be fertilized on removal to sea water after 40 minutes in the solutions and produced normal plutei. However, the eggs cannot be fertilized while in the solutions; the sperm are immotile in all except NaCl, and here no fertilization membrane was seen.

Arbacia punctulata eggs were placed in 50 cc. of the isosmotic salt solution for 20 minutes and this was replaced by a fresh salt solution for another 20 minutes. Three tubes of experimental eggs (in different salt solutions) and one tube of control eggs (in sea water) were centrifuged at the same time; isosmotic sugar solution was placed in the bottom of each tube to keep the eggs suspended. Care must be taken that exactly the same amount of sugar solution is used in each tube and exactly the same amount of egg suspension placed on top, so that the eggs in each tube are thrown to the same level and are subjected to exactly the same amount of centrifugal force. For stratification the force used was about 3,000 × g for two minutes, and for breaking 10,000 × g for four minutes. Each experiment was repeated many times. A single batch of eggs was always used in each experiment.

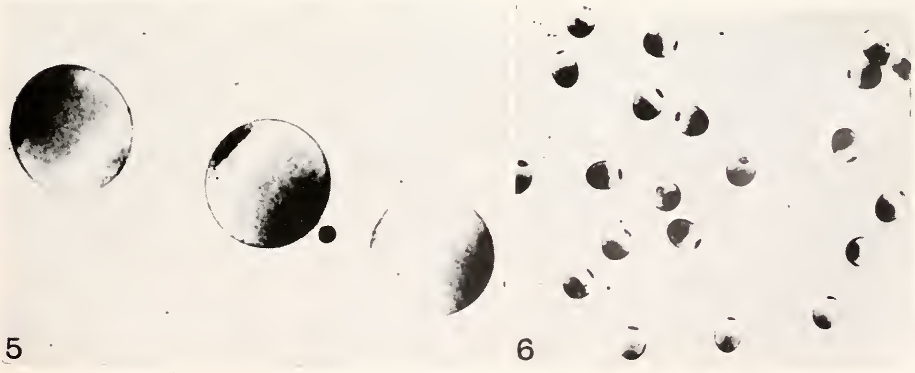
Stratification NaCl (KCl) Breaking



Sea Water



$MgCl_2$ ($CaCl_2$)



EXPLANATION OF PLATE

Stratification of *Arbacia punctulata* eggs centrifuged at 3,000 × g for two minutes in (1) NaCl, (3) sea water, (5) $MgCl_2$. Breaking apart of eggs at 10,000 × g for four minutes in (2) NaCl, (4) sea water, (6) $MgCl_2$. KCl acts much like NaCl and $CaCl_2$ much like $MgCl_2$.

The experiments were carried out at approximately 23° C., so that the temperature effect observed by Costello (1934, 1938) was not involved.

It was found that in the monovalent salts, NaCl and KCl, the rate of stratification is less than in sea water, and in the bivalent salts, $CaCl_2$ and $MgCl_2$, the rate of stratification is greater than in sea water (Photographs 1, 3, 5). The viscosity, then, is increased in NaCl and KCl and decreased in $CaCl_2$ and $MgCl_2$. In the effect on the rate of stratification the series runs, from most to least: Ca > Mg > S.W. > Na > K. This is similar to the series given by Heilbrunn (1923, 1928) in a slightly different experiment with *Arbacia* eggs, except that Na and K are reversed. This is possibly due to a difference in the tonicity of the solutions used. His series for *Stentor* is the same as my series for *Arbacia*.

In ease of breaking with centrifugal force, the series runs in the reverse order. Eggs in KCl, where the stratification is least in a given time, break most readily, and those in $CaCl_2$, where the stratification is greatest, break least readily. Eggs in the monovalent salts, NaCl and KCl, break more readily than those in sea water while the eggs in the bivalent salts, $MgCl_2$ and $CaCl_2$, break less readily than those in sea water (Photographs 2, 4, 6). In ease of breaking, the series runs, from greatest to least: K > Na > S.W. > Mg > Ca. The ease of breaking has been judged by the percentage of eggs broken in a given time with a constant force, rather than by the time for a definite percentage to break, since the experiment can be carried out more accurately when experimental and control eggs are centrifuged at the same time. An average experiment gives the following figures for percentage of eggs broken when centrifuged for four minutes at 10,000 × g.

KCl	NaCl	Sea water	$MgCl_2$	$CaCl_2$
99%	90%	50%	20%	none

There was no measurable difference in the relative size of the two "halves" in any of the pure salt solutions; the white and red "halves" were the same size as those obtained when eggs were kept and centrifuged in sea water.

There is considerable variation in ease of breaking in different lots of eggs with the same centrifugal force, and even the same batch varies slightly after being kept in sea water for several hours. In one experiment 98 per cent were broken in sea water, and 40 per cent in $CaCl_2$; in another experiment, 50 per cent were broken in NaCl and 20 per cent in sea water. In every experiment, however, the eggs in the solutions broke in the order named. It was thought that possibly the jelly surrounding the eggs might be influenced by the salt solutions and be responsible for the difference in ease of breaking. Jelly was found to be present on the eggs in all the solutions. Eggs from which the jelly was removed by addition of 0.2 cc. N/10 HCl to 50 cc. sea water, and then well washed in sea water broke in the solutions in the same order as those with jelly.

Since the experimental results are contrary to the expectation that the interior viscosity is the controlling factor in breaking of the eggs, we are led to the conclusion that the salts affect the "tension at the surface." Despite the increased interior viscosity in pure NaCl and KCl, the surface forces resisting the pulling apart of the eggs are actually decreased. In $CaCl_2$ and $MgCl_2$ they are increased though the interior viscosity is decreased. Heilbrunn (1923, 1943) has pointed out that in *Amoeba*, and apparently also in *Arbacia* eggs, the cortical protoplasm reacts differently from the interior protoplasm, and Brown (1934) has found a difference in cortical and interior protoplasm in response to hydrostatic pressure on fertilized *Arbacia* eggs.

An effect on the surface forces without any effect on the interior viscosity is given by eggs in Ca-free sea water. Unfertilized eggs kept and centrifuged in Ca-free sea water stratify at the same rate as those in sea water, as shown in previous experiments with a double image centrifuge microscope (E. B. Harvey, 1933). They break apart more readily in Ca-free sea water than in normal sea water—at about the same rate as those in NaCl alone. The fertilized eggs also break more readily in Ca-free sea water than in normal sea water, as shown previously. The absence of calcium therefore tends to decrease the surface forces and the presence of calcium alone tends to increase them. That calcium has an effect on the surface layers of eggs is well known, and has been especially emphasized by Heilbrunn (1928, 1943). A very good example is given by the classic experiments of Herbst (1900) in separating blastomeres due to the dissolution of the ectoplasmic (hyaline plasma) layer in Ca-free sea water.

SUMMARY

When unfertilized *Arbacia punctulata* eggs are centrifuged in isosmotic single salt solutions, they stratify with decreasing readiness (indicating increasing viscosity) in the following order: $CaCl_2 > MgCl_2 > S.W. > NaCl > KCl$. They break into "halves" with decreasing ease in the reverse order, those in $CaCl_2$ which stratify best, break least readily. In the bivalent salts they stratify better and break less readily than in sea water, and in the monovalent salts they stratify less and break more readily than in sea water. The ease of breaking must be determined by an effect of the salts on the surface layers rather than by their effect on the interior viscosity.

LITERATURE CITED

Barth, L. G., 1929. The effects of acids and alkalies on the viscosity of protoplasm. *Protoplasma*, **7**: 505–534.

Brown, D. E. S., 1934. The pressure coefficient of "viscosity" in the eggs of Arbacia punctulata. *Jour. Cell. and Comp. Physiol.*, **5**: 335–346.

Cole, K. S., 1932. Surface forces of the Arbacia egg. *Jour. Cell. and Comp. Physiol.*, **1**: 1–9.

Costello, D. P., 1934. The effects of temperature on the viscosity of Arbacia egg protoplasm. *Jour. Cell. and Comp. Physiol.*, **4**: 421–433.

Costello, D. P., 1938. The effect of temperature on the rate of fragmentation of Arbacia eggs subjected to centrifugal force. *Jour. Cell. and Comp. Physiol.*, **11**: 301–307.

Harvey, E. B., 1933. Effects of centrifugal force on fertilized eggs of Arbacia punctulata, as observed with the centrifuge microscope. *Biol. Bull.*, **65**: 389–396.

Harvey, E. B., 1943. Rate of breaking and size of the "halves" of the Arbacia punctulata eggs when centrifuged in hypo- and hypertonic sea water. *Biol. Bull.*, **85**: 141–150.

Heilbrunn, L. V., 1923. The colloid chemistry of protoplasm. I. General considerations. *Amer. Jour. Physiol.*, **64**: 481–489.

Heilbrunn, L. V., 1928. *The colloid chemistry of protoplasm.* Monograph. Berlin.

Heilbrunn, L. V., 1943. *An outline of general physiology*, 2nd edition. Saunders Co.

Herbst, C., 1900. Über das Auseinandergehen von Furschungs- und Gewebzellen in kalkfreiem Medium. *Arch. f. Entw. Mech.*, **9**: 424–463.

THE EFFECT OF CYANIDE ON RESPIRATION IN PARAMECIUM CAUDATUM AND PARAMECIUM AURELIA[1]

D. M. PACE

Department of Physiology and Pharmacology, College of Pharmacy,
University of Nebraska, Lincoln, Nebraska

In some ciliates the presence of a cytochrome-oxidase system has been established. Pitts (1932) claimed that *Colpidium campylum* showed an ititial sensitivity to HCN but that the oxygen consumption soon increased until it even surpassed normal consumption. Lwoff (1934) also found an initial inhibition followed by an acceleration in respiration in another ciliate, *Glaucoma pyriformis,* when it was exposed to KCN. Hall (1941) definitely established that HCN inhibits respiration in *Colpidium campylum* and Baker and Baumberger (1941) found that HCN inhibits respiration in *Tetrahymena geleii.*

Paramecium is usually cited as one of the several exceptions to the rule that most animal cells are sensitive to HCN. In fact, ciliates as a group have been regarded by some investigators as being insensitive to cyanide, although very few species have been tested. Lund (1918), Shoup and Boykin (1931), and Gerard and Hyman (1931) found that *Paramecium caudatum* was resistant to cyanide. However, Child (1941) refers to unpublished data obtained by Hyman, in which she found a considerable decrease in O_2 consumption of *Paramecium* in KCN. Dr. Hyman[2] has also informed the author by personal communication that she

---

[1] These investigations were partly supported by a grant-in-aid received from the Society of Sigma Xi. The Barcroft-Warburg apparatus was purchased by a grant furnished by Mr. Arthur S. Raymond of the Lincoln Drug Co., Lincoln, Nebraska.

[2] Dr. Libbie H. Hyman has granted me the privilege of using the following communication which she sent to me at my request: "Some years ago, being skeptical of Lund's failure to find any cyanide-sensitive respiration in Paramecium, I spent a great deal of time and effort in testing the action of cyanide on the oxygen consumption of Paramecium, using Winkler's method. I met with so many difficulties that I never published the results; chief among them were the impossibility of measuring equal suspensions of Paramecium from a volumetric pipette because the animals adhere to the glass, and the toxicity to Paramecium of all waters except the culture water, which in itself has high oxygen consuming powers. However, my results indicated that starved Paramecium have no cyanide-sensitive respiration, in agreement with the finding of Lund, but non-starved ones have about 35 per cent such respiration. After giving up the work as impractical by my methods, I sought the help of Dr. Gerard. Dr. Gerard kindly consented to test the matter on his manometers but failed to find any depressing action of cyanide on non-starved Paramecium. As I played no role in this work except that I furnished the Paramecium, I feel that Dr. Gerard was over-generous in making me co-author. I was not satisfied with these results, first, because successive manometric readings were highly variable, and second, because the buffer solution used was toxic to Paramecium, depressing oxygen consumption by about 50 per cent in itself.

"As a cyanide sensitivity of the extra oxygen consumption caused by feeding was indicated in my experiments, it became interesting to know the nature of this extra respiration. I therefore attempted to compare the effects on oxygen consumption of the ingestion by Paramecium of particles without food value (carbon suspension) and of particles with food value (yeast). Here, again, I met with insuperable difficulties. I could never get any sample of yeast, no found an inhibition of O_2 consumption in *P. caudatum* when it was exposed to HCN.

Sato and Tamiya (1937) claimed that they found cytochrome a and c in *Paramecium*. If this is true, then it is difficult to understand the insensitivity of the respiratory mechanism of this species to HCN. Because of these observations and of the unpublished results of Hyman, and since studies have not been made on the sensitivity of Paramecium to cyanide when proper KOH–KCN mixtures are used as absorption media (Krebs, 1935), the following investigation was carried out.

MATERIAL AND METHODS

Two species were used in this work, *Paramecium caudatum* and *Paramecium aurelia*. The culture solution used was highly buffered and was the same as was used later in the flasks of the Barcroft-Warburg apparatus for testing. The solution consisted of $K_2HPO_4 . H_2O$ — 80 mg., KH_2PO_4 — 80 mg., $CaCl_2$ — 104 mg., Mg_3PO_4 — 2 mg., and redistilled water to make one liter.

In making up the stock culture, 15 gms. of timothy hay were boiled in 500 ml. of this solution for one-half hour, after which the solution was made up to its original volume by the addition of distilled water. This "broth" was then diluted further by the addition of the above buffered solution to make 4000 ml. The hydrogen ion concentration was held at pH 7.0 ± 0.2.

This culture solution, along with approximately 3 gms. of sterile hay, was put into chemical bottles with 500 ml. capacity and moderately narrow necks (3–4 cm. in diameter). About 4000 paramecia were added to each container. Within 5 days they became extremely numerous, especially in the neck region of the bottle whence they could be removed easily in large numbers.

The Barcroft-Warburg apparatus was used for ascertaining rate of oxygen consumption. The shaking mechanism was adjusted to operate at 110 complete cycles per minute. Because of the possibility of NH_3 production (Specht, 1934), a 0.3 ml. portion of 0.3 N HCl was added to the side arm (onset) of each manometer flask.

During the course of these investigations, various test solutions were made up containing different concentrations of KCN as follows: 0, 10^{-5}, 10^{-4}, and 10^{-3} M. Corresponding KOH–KCN absorption solutions were made up for each concentration of test solution according to Krebs (1935), and 0.4 ml. of the proper mixture (Pace and Belda, 1944) was added to the inner well (inset) of each flask containing organisms in KCN. To the inset of each of the flasks in which the test solution contained no KCN, a 0.4 ml. portion of M KOH was added.

A typical test was made in the following manner: Paramecia were drawn off from the top of the bottles in which they were cultured and placed in 15 ml. centrifuge tubes in which they were washed several times in fresh solution by careful centrifugation. The only time the organisms were subjected to centrifugation was

matter how many times boiled and centrifuged, that did not have high oxygen consuming powers, and all carbon suspensions also remove oxygen from the medium. However, there were indications that ingestion of a non-nutritive substance can cause as great an increase in oxygen consumption as does ingestion of food. This suggests that the extra respiration of feeding does not result from an oxidation of the food material."

during the washing process and this was carried out with great care by means of a hand centrifuge. An attempt was made to have between 2000 and 3000 *P. aurelia* or 1000 and 2000 *P. caudatum* in each 5 ml. sample. A count was always made of the organisms in each flask at the end of an experiment.

In all the tests reported here, those organisms designated "young" paramecia were taken from 5–7 day-old cultures; those designated "old" paramecia, from 15–20 day-old cultures; those designated "starved" paramecia were "old" organisms that had been placed in inorganic buffer solution without food material for 2 or 3 days. The "young" paramecia had much more food material present in the form of food vacuoles than the "old" paramecia.

RESULTS

Effect of cyanide on respiration in Paramecium aurelia

Paramecium aurelia was the first species studied. It is a much smaller form than *P. caudatum*, but its rate of respiration per unit volume is similar to the latter (Pace and Kimura, 1944).

A number of tests were made at various KCN concentrations. Organisms that were taken from cultures 15–17 days after they had been started (i.e., "old" paramecia) were used in most of the tests. They were washed by centrifugation in the solution given above, and then divided into two portions. KCN was added to one of these portions in the concentrations designated in the table. Several tests were also carried out on starved paramecia and young paramecia. The results are presented in Table I.

P. aurelia was found to be sensitive to KCN in all the tests made, except where starved individuals were used. The normal average oxygen consumption for organisms taken from the 15 or 17 day-old cultures was 6.31 mm^3 per hour per mm^3 of cell substance at 25° C. This compares favorably with the results of Pace and Kimura (1944) who found that *P. aurelia* consumed oxygen at the rate of 6.16 mm^3 per hour per mm^3 of cell substance at 25° C.

The presence of food material may have something to do with the fact that in all the tests made, the younger paramecia showed a much greater sensitivity to cyanide than the older. In fact, starved specimens were insensitive to cyanide. When exposed to KCN at a concentration of 10^{-4} M, respiration in the young organisms was inhibited on the average by about 40 per cent. The respiration of old organisms showed an average inhibition of 22 per cent to the same concentration of KCN. At KCN concentrations of 10^{-3} M, inhibition of respiration was greater than with the lower concentration, but the results were similar insofar as young and old organisms are concerned. In young paramecia, the average O_2 consumption (1318 mm^3 O_2 per hour per million) in the buffered solution without KCN was about twice that in old organisms. An average O_2 consumption of 640 mm^3 was found for the young paramecia when they were exposed to 10^{-3} M KCN. Thus the cyanide at this concentration results in a 50 per cent inhibition in respiration in *P. aurelia*.

Effect of KCN on respiration in Paramecium caudatum

Paramecium caudatum has been studied to a much greater extent than *P. aurelia* and, as brought out previously, all the work (except for unpublished early

TABLE I

The effect of KCN on respiration in *Paramecium aurelia*. *, starved specimens; 5–7 day cultures, young specimens; all others, old specimens. Temperature, 25° C.; pH, 7.0 ± 0.2. Average volume of one million paramecia, 121.4 mm.^{3} (this does not include the volume of starved specimens). Each figure in columns 4 and 5 represents the average for 3 tests.

Molar concentration of KCN	Age of culture in days	Duration of test in hours	Average O_2 consumption in mm.^{3} per hour per million	Average O_2 consumption in mm.^{3} per hour per mm.^{3} of cell substance	Per cent inhibition
0 10^{-4}	17*	4	462 484		None
0 10^{-4}	16	3	746 598	6.14 4.92	19.9
0 10^{-4}	16	3	680 485	5.60 3.99	29
0 10^{-4}	15	5	709 453	5.84 3.73	36.1
0 10^{-4}	15	3	808 665	6.65 5.47	18
0 10^{-4}	15	3	841 747	6.92 6.15	12
0 10^{-4}	7	3	906 657	7.46 5.42	28.5
0 10^{-4}	5	5	1360 788	11.20 6.49	42
0 10^{-3}	16*	3	520 511		None
0 10^{-3}	15	3	818 557	6.73 4.58	32
0 10^{-3}	5	3	1516 605	12.48 4.98	60
0 10^{-3}	6	5	1120 677	9.22 5.57	40

results of Dr. Libbie H. Hyman) indicates that *P. caudatum* is insensitive to cyanide. One great difference in the work reported here and previous investigations carried out on the effect of cyanide on *Paramecium* is that in these experiments suitable KCN–KOH absorption mixtures rather than pure KOH were used in the manometer flasks to prevent absorption of HCN from the test solution.

The same procedures were followed here as for *P. aurelia*. The results are presented in Table II.

As indicated by the results, much variation was found in the action of KCN on *Paramecium caudatum*. In the first few tests very great difficulty was experi-

TABLE II

The effect of KCN on oxygen consumption in *Paramecium caudatum*. *, starved specimens; 5 day cultures, young specimens; all others, old specimens. Temperature, 25° C.; pH, 7.0 ± 0.2. Average volume of one million paramecia, 591 $mm.^3$ Each figure in columns 4 and 5 represents the average for 3 tests.

Molar concentration of KCN	Age of culture in days	Duration of test in hours	Average O_2 consumption in $mm.^3$ per hour per million	Average O_2 consumption in $mm.^3$ per hour per $mm.^3$ of cell substance	Per cent inhibition
0 10^{-5}	16*	3	1565 1518		None
0 10^{-5}	16	2	3273 2734	5.53 4.62	15.5
0 10^{-5}	16	6	3734 3181	6.33 5.37	15
0 10^{-5}	5	9	4420 2650	7.47 4.48	30
0 10^{-4}	17	3	3040 3010	5.14 5.09	None
0 10^{-4}	19	5	2700 1978	4.56 3.34	27
0 10^{-4}	15	3	3787 2243	6.40 3.80	40
0 10^{-4}	5	4	4270 2475	7.22 4.18	42
0 10^{-3}	16*	5	1190 1280		None
0 10^{-3}	15	3	3580 2072	6.05 3.50	42
0 10^{-3}	5	12	4590 1560	7.76 2.63	66
0 10^{-3}	15	4	4170 2380	7.05 4.02	43

enced, chiefly because some apparently minor details in manipulation were overlooked and this may have had a very noticeable effect on the results. It was suspected from the results of the first few tests that food played an important part in the degree of sensitivity of these organisms to KCN. For this reason several tests were conducted on this species under the same type of conditions as was used for *P. aurelia,* namely: (1) young paramecia (5 day cultures), (2) old paramecia (15 to 19 day cultures) and (3) starved paramecia.

The results indicate that although there was great variation in some of them, the young specimens show a greater sensitivity to KCN. The starved specimens proved to be non-sensitive. In some tests there appeared to be an actual acceleration of O_2 consumption when starved *P. caudatum* was put into KCN solutions but the results may have been due to experimental error. They are not included in the table. In one test (included in table) which was made upon old organisms, there was no evidence of cyanide sensitivity; no explanation can be given for this exception.

The average inhibition of O_2 consumption found in old *P. caudatum* exposed to solutions containing 10^{-5} M KCN was approximately 15 per cent; in solutions containing 10^{-4} M, 33 per cent; and in solutions containing 10^{-3} M, 42 per cent. In young *P. caudatum* exposed to 10^{-5} M KCN, respiratory inhibition was approximately 30 per cent; in solutions containing 10^{-4} M KCN, 42 per cent; and in solutions containing 10^{-3} M, approximately 66 per cent. Thus, inhibition of oxidative metabolism increases with increase in KCN concentration, and the degree of sensitivity to cyanide seems to depend upon the quantity of food material present. This is in agreement with the results of Hyman. Higher concentrations than 10^{-3} M KCN were attempted but the results are meaningless because of such extreme variations and for this reason they have not been included in this report.

Effect of dextrose on the degree of inhibition by cyanide

Many workers have reported that one of the factors in the sensitivity of the respiratory mechanism to cyanide is the degree of carbohydrate saturation in the cell. Keilin (1932) suggests that perhaps the most important factor concerned with cellular sensitivity to cyanide is the concentration of carbohydrate. Commoner (1939) working with bakers' yeast, Emerson (1927) with *Chlorella*, and Hall (1941) with *Colpidium campylum*, all found either a greater inhibition with cyanide when dextrose was present or no inhibition without dextrose.

Since it is highly probable that a large portion of the food material of *Paramecium* is carbohydrate and since it was found that the greatest sensitivity to cyanide occurred when the greatest quantity of food was present, it was thought advisable to run respiration tests with the organisms in a dextrose solution.

Old paramecia were selected and washed in the buffered test solution containing 0.01 M dextrose. Then the solution containing the paramecia was divided

Table III

The effect of KCN on *Paramecium caudatum* in a 0.01M dextrose-buffer solution. All the organisms were taken from 16 to 19 day-old stock cultures. Temperature, 25° C.; pH, 7.0 ± 0.2. Average volume of one million paramecia, 580 mm.³ Each figure represents the average for 3 tests.

Molar concentration of KCN	Age of culture in days	Duration of test in hours	Average O_2 consumption in mm.³ per hour per million	Average O_2 consumption in mm.³ per hour per mm.³ of cell substance	Per cent inhibition
0	16	4	4550	7.84	48
10^{-4}			2360	4.06	
0	16	5	3860	6.65	51
10^{-4}			1890	3.25	
0	19	3	4120	7.10	54
10^{-4}			1895	3.26	

into two portions. To one portion, KCN was added to 10^{-4} M; the other portion was used as control. This experiment was repeated twice and the results are presented in Table III.

The results show that the rate of respiration in *Paramecium caudatum* is increased with the addition of dextrose to the test solution. The average rate of respiration in the dextrose-buffer solution for all tests without KCN added was 4170 mm^3 per hour per million organisms as compared to an average 3470 mm^3 in the same type of organisms tested in the buffer solution without dextrose (Table II). They also show that there was an average inhibition of 51 per cent in O_2 consumption in 10^{-4} M KCN in the dextrose-buffer solution which is much greater than the average inhibition in 10^{-4} KCN without dextrose. The average inhibition for two experiments in which the latter solution was used, was 33.5 per cent; in one of the experiments there was no inhibition whatever, but this has not been included in the average.

DISCUSSION

Many factors may have contributed to the failure of earlier investigators to find inhibition in respiration in *Paramecium* when exposed to cyanide. Considerable error must have been caused by the absorption of free HCN by the KOH used as absorption fluid. The initial inhibitory effect followed by an increase in oxygen consumption noted in the results of Pitts (1932) and Lwoff (1934) is evidently due to the fact that little attention was given to the rapid absorption of cyanide (via distillation of HCN) by the absorption fluid. Hall (1941), using suitable KOH–KCN mixtures as absorption media, proved conclusively that respiration in *Colpidium* was cyanide sensitive.

In the investigations reported here, care was taken to prevent distillation of HCN over into the absorption fluid. However, there is another factor that may or may not have been realized by these earlier workers, namely, the food content of the paramecia with which they worked. It is possible that the organisms used by them were taken from "old" cultures and hence had comparatively little food material in them. If this be true, it explains their failure to find inhibition in respiration, for, as reported above, sensitivity seems to depend, at least partly, upon the food content of *Paramecium*. This very important factor was noted some twenty years ago by Dr. Libbie Hyman (see footnote 2).

In these experiments, the organisms were taken from the culture solution, washed, and placed in fresh test solution, and then put into manometer flasks, all within 10–15 minutes. Thus in most of the tests the organisms were actually in inorganic solution without food for 3.5 hours; in some tests 4.5–5.5 hours, but rarely longer than this. During this time, very little change could be noted in food vacuole content or size. It was also noted that respiration varied very little, if at all, from the beginning to the end of a test. In other words, the decrease in food content is so slight within this short period of time that there was no noticeable change in rate of respiration.

Carbohydrate makes up a great portion of the food of *Paramecium*. One of the most important factors in the degree of sensitivity of respiration to KCN, etc. is the concentration of carbohydrate in the cell. Thus when dextrose was added to the buffer solution in which the respiration of *Paramecium caudatum* was tested, the per cent inhibition was greater than in the buffer solution without dextrose.

SUMMARY

1. The oxygen consumption in *Paramecium caudatum* and *Paramecium aurelia* is partially inhibited by potassium cyanide.

2. The extent of inhibition by cyanide is dependent upon the food content of the organisms as well as upon the concentration of cyanide in the solution.

3. In *P. aurelia*, starved specimens are insensitive to cyanide; old specimens are not as sensitive as young. In 10^{-4} M KCN respiration in the old organisms was inhibited by approximately 22 per cent while in the young organisms it was inhibited by approximately 40 per cent.

4. In *Paramecium caudatum*, starved specimens were non-sensitive to KCN; old specimens exposed to 10^{-3}, 10^{-4}, and 10^{-5} M KCN show, respectively, a 42, 33, and 15 per cent inhibition in respiration. Young specimens, exposed to 10^{-3}, 10^{-4}, and 10^{-5} M KCN show, respectively, a 66, 42, and 30 per cent inhibition.

5. The inhibition in the rate of respiration in *P. caudatum* was greater in buffer solution plus dextrose (0.01 M) than in the same solution without dextrose.

6. The effect of cyanide on respiration in *Paramecium* depends upon the degree of saturation of the respiratory mechanism with carbohydrate.

LITERATURE CITED

Baker, E. G. S., and Baumberger, J. P., 1941. The respiratory rate and the cytochrome content of a ciliate protozoan (Tetrahymena geleii). *J. Cell. and Comp. Physiol.*, **17**: 285–303.

Child, C. M., 1941. Patterns and Problems of Development. University of Chicago Press, Chicago, Ill. 811 pp.

Commoner, B., 1939. The effect of cyanide on the respiration of bakers' yeast in various concentrations of dextrose. *J. Cell. and Comp. Physiol.*, **13**: 121–138.

Emerson, R., 1927. The effect of certain respiratory inhibitors on the respiration of Chlorella. *J. Gen. Physiol.*, **10**: 469–477.

Gerard, R. W., and Hyman, L. H., 1931. The cyanide sensitivity of Paramecium. *Amer. J. Physiol.*, **97**: 524–525.

Hall, R. H., 1941. The effect of cyanide on oxygen consumption of Colpidium campylum. *Physiol. Zool.*, **14**: 193–208.

Keilin, D., 1932. Cytochrome and intracellular respiratory enzymes. *Ergeb. der Enzymforschung*, Bd. **2**: 239–271.

Krebs, H. A., 1935. Metabolism of amino-acids. III. Deamination of amino acids. *Biochem. J.*, **29**: 1620–1644.

Lund, E. J., 1918. Rate of oxidation in P. caudatum and its independence of the toxic action of KCN. *Amer. J. Physiol.*, **45**: 365–373.

Lwoff, M., 1934. Sur la respiration du Cilie Glaucoma piriformis. *C. R. Soc. Biol., Paris*, **115**: 237–241.

Pace, D. M., and Belda, W. H., 1944. The effects of potassium cyanide, potassium arsenite, and ethyl urethane on respiration in Pelomyxa carolinensis. *Biol. Bull.*, **87**: 138–144.

Pace, D. M., and Kimura, K. K., 1944. Effect of temperature on respiration in Paramecium caudatum and Paramecium aurelia. *J. Cell. and Comp. Physiol.*, **24**: 173–183.

Pitts, R. F., 1932. Effect of cyanide on respiration of the protozoan, Colpidium campylum. *Proc. Soc. Exp. Biol. N. Y.*, **29**: 542.

Sato, T., and Tamiya, H., 1937. Über die Atmungsfarbstoffe von Paramecium. Cytologia. *Fujii Jubilee Volume*, pp. 1133–1138.

Shoup, C. S., and Boykin, J. T., 1931. The sensitivity of Paramecium to cyanide and effects of iron on respiration. *J. Gen. Physiol.*, **15**: 107–118.

Spacht, H., 1934. Aerobic respiration in Spirostomum ambiguum and the production of ammonia. *J. Cell. and Comp. Physiol.*, **5**: 319–333.

THE AGGLUTINATION OF STARFISH SPERM BY FERTILIZIN[1]

CHARLES B. METZ[2]

*William G. Kerckhoff Laboratories of the Biological Sciences,
California Institute of Technology, Pasadena, California*

Agglutination of starfish sperm by specific egg water (supernatant sea water from egg suspensions) has never been clearly demonstrated. Glaser (1914) and Woodward (1918) reported a strong agglutination of *Asterias forbesii* sperm by homologous egg water, but Just (1930) was unable to confirm this work. Attempts to demonstrate agglutination of sperm by egg water in other species of starfish have failed. Thus Loeb (1914) observed no reaction in *Asterias* (probably *Pisaster*) *ochraceus*, and Tyler (1941) had a similar result with *Patiria miniata*. From this it might appear that fertilizin is not present in starfish egg water. However, Tyler found that treatment of *Patiria* sperm with egg water lowered the fertilizing power of the sperm. Tyler (1941, 1942) interpreted this as support for his view that fertilizin may exist naturally in a non-agglutinating "univalent" form. An individual molecule of such univalent fertilizin should have but one combining group capable of reacting with groups (antifertilizin) on the sperm surface. On the basis of the Marrack-Heidelberger (1938) lattice theory, univalent fertilizin should therefore combine with but not agglutinate these cells. Tyler suggests that such univalent fertilizin may be present quite generally in forms showing no agglutination of sperm by egg water. He therefore supports the belief held by Lillie (1919) and Just (1930) that fertilizin occurs universally.

In view of the concept of univalent fertilizin and the provisional status of the starfish with respect to sperm agglutination by egg water, it is of some interest that sperm of certain starfish agglutinate when mixed with homologous egg water and an "adjuvant." The first adjuvant found was lobster (*Panulirus*) serum. The agglutination reaction was discovered accidentally in the course of studies on the natural agglutinins in lobster serum (Tyler and Metz, 1944). In an attempt to separate natural agglutinins for *Patiria* eggs and sperm, the serum was treated with eggs and then titrated for sperm agglutinins. The treatment with eggs increased the sperm agglutinin titer several fold. Investigation of this unexpected result showed that sperm absorbed lobster serum (freed of natural sperm agglutinins), when mixed with *Patiria* egg water, agglutinated *Patiria* sperm. Tests on other material showed the presence of adjuvant in hen's egg white. A preliminary report (Metz, 1944) on this work has already appeared. The studies confirm Tyler's view that fertilizin is present in *Patiria* egg water. However, the experiments indicate that this fertilizin is multivalent. Data are given which suggest that normal *Patiria* sperm is "univalent" with respect to exposed antifertilizin groups, but that more of these groups are "exposed" by the adjuvant.

---

[1] Submitted to the Graduate School of the California Institute of Technology in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Present address: Shanklin Laboratory of Biology, Wesleyan University, Middletown, Conn.

MATERIAL AND METHODS

The Pacific webbed star, *Patiria miniata*, was used as standard material. The Pacific star *Pisaster ochraceus*, the Pacific sand star *Astropecten* sp. and the Atlantic *Asterias forbesii* were used in confirmatory and specificity tests.

Egg and sperm suspensions were prepared from ripe extirpated gonads. These organs were minced in a measured volume of sea water and then filtered through bolting cloth to remove the gonadal tissue. The difference in volume of the filtrate and the sea water initially added gives the volume of "dry" (undiluted) material. Egg and sperm dilutions were reckoned from this "dry" volume. Egg water solutions were obtained by drawing off the supernatant from standing egg suspensions (25–50 per cent of dry eggs in sea water), or by heating such suspensions and filtering or centrifuging off the eggs.

Lobster (*Panulirus interruptus*) serum was obtained by drawing blood from the heart and allowing it to clot. After syneresis the serum was drawn off. Since *Panulirus* serum contains natural heteroagglutinins for sperm of various organisms (Tyler and Metz, 1944) including *Patiria, Pisaster* and *Astropecten*, it is impractical to use the untreated serum. By absorption with *Patiria* sperm the natural agglutinins for *Pisaster* and *Astropecten* as well as *Patiria* sperm can be removed. Such absorbed serum was used as the adjuvant for sperm of all three species. For reasons of economy both in material and time, hen's egg white was used as the adjuvant in the later experiments. This material was made isotonic by adding one volume of concentrated (1.73 ×) sea water. It was then diluted to 20 per cent with normal sea water and filtered to remove the mucin, chalazae and other insoluble material. This diluted egg white was usually heated to 100° C. and filtered or centrifuged since this procedure increased its activity several fold. Hen's egg white does not contain natural agglutinins for *Patiria, Pisaster* or *Astropecten* sperm. Thus, initial absorption with starfish sperm was not necessary.

Assays of unknown egg water were made by diluting the unknown solution in twofold steps with sea water and then adding constant amounts of adjuvant-treated sperm to each dilution of unknown egg water. Adjuvant was titrated in a similar manner. However, when titrating adjuvant, constant amounts of sperm suspension were added to the dilutions of unknown adjuvant. Subsequently, constant amounts of egg water were added to each adjuvant dilution. Less satisfactory results are obtained if any other order of mixing is employed in this test. In all cases the presence or absence of agglutination was determined by microscopical examination one to several minutes after mixing. Titers were recorded as the highest dilution of unknown showing agglutination of the test sperm.

The apparatus and methods used in ultraviolet irradiation have been described in a previous article (Metz, 1942).

ACTIVATION AND AGGLUTINATION OF STARFISH SPERM

In these studies no definite agglutination of *Patiria, Pisaster, Astropecten* or *Asterias* sperm was observed following the addition of homologous egg water. However, *Patiria* sperm suspensions frequently appeared "granular" after this treatment. These microscopic "granules" consisted of two or three sperm fixed together and represent a plus-minus agglutination reaction. Various devices such as centrifugation were employed in an attempt to bring this reaction to a distinct agglutination, but all of them failed.

The starfish sperm in dilute (0.5 to 1.0 per cent) sea water suspension were virtually immobile. The cells did not respond to treatment with fresh sea water (lowering CO_2 tension) or with homologous egg water. Starfish sperm thus differ from *Arbacia* and *Nereis* sperm which become more active when diluted with sea water (Lillie, 1913; Just, 1930), and from *Arbacia* (Lillie, 1913), *Strongylocentrotus* (Tyler, 1939) and *Megathura* sperm (Tyler, 1940) which become intensely motile when mixed with homologous egg water.

Patiria, Pisaster and *Astropecten* sperm, although refractory to treatment with sea water and egg water, nevertheless became intensely active when treated with isotonic hen's egg white or the serum of the lobster (*Panulirus*), fish (*Crassius*), hen, or rabbit. Furthermore, adjuvant-treated sperm of these starfish agglutinated strongly on addition of homologous egg water. *Asterias* was tested on three successive seasons. The sperm became intensely active when treated with isotonic hen's egg white. Weak agglutination sometimes occurred after addition of homologous egg water to the sperm egg white suspension. Unfortunately, the agglutination was so weak and occurred so irregularly that quantitative studies could not be made.

In *Patiria* the agglutination resulting from treatment of sperm with adjuvant and egg water was exclusively head to head. Each clump consisted of a central mass of sperm heads tightly bound together, and a peripheral region of free tails which projected out radially from the central mass of heads. *Patiria* thus differs from *Megathura,* since sperm of the latter agglutinate tail to tail as well as head to head (Tyler, 1940). The clumped *Patiria* sperm soon became immobile even though the free sperm remained active for an hour or more. The spontaneous reversal of agglutination so characteristic of the sea urchin occurred to a limited extent only after the free sperm had become inactive.

Properties of Patiria Fertilizin

Fertilizin may be defined by the following properties: (1) it combines with (but does not necessarily agglutinate) sperm, (2) it is highly specific in this reaction, and (3) it is obtained primarily from eggs. Studies on the role of egg water in the agglutination of treated sperm show that *Patiria* egg water has these properties.

Absorption of Patiria egg water by sperm. A direct combination between sea urchin fertilizin and sperm may be demonstrated by absorption of egg water with sperm, or by neutralization of egg water with appropriate sperm extract (Lillie, 1913; Frank, 1939). Similarly, it may be shown that sperm-absorbed *Patiria* egg water will no longer agglutinate treated sperm. Indeed, complete exhaustion of the egg water may be attained even in the absence of adjuvant. In a typical experiment 20 drops of *Patiria* egg water were mixed with 22 drops of concentrated (25–50 per cent) *Patiria* sperm. The mixture was set aside to allow for reaction. Twenty drops of the same egg water were mixed with 22 drops of sea water to serve as a control. After centrifugation the fluid of both tubes was titrated with adjuvant-treated (0.5–1 per cent) sperm. The undiluted absorbed egg water did not agglutinate the sperm, whereas the control unabsorbed egg water clumped the sperm even at a dilution of $\frac{1}{256}$ of full strength. Other controls showed that sperm without adjuvant were not agglutinated by control or absorbed egg water, or by the adjuvant (*Patiria* sperm-absorbed *Panulirus* serum). Thus a substance (fertilizin) is present in *Patiria* egg water which will combine with specific sperm independently of the adjuvant. The adjuvant is required only for agglutination.

Specificity of starfish fertilizin. The reaction between sperm and fertilizin is characterized by a high order of specificity (Tyler, 1940). Cross tests between *Patiria, Pisaster* and *Astropecten* sperm and fertilizin show that these starfish are not exceptional in this respect. Sperm suspensions of the three species were treated with *Patiria* sperm-absorbed *Panulirus* serum, and then cross tested with the egg waters of the three species. The data are given in Table I.

TABLE I

Specificity of Patiria, Pisaster *and* Astropecten *egg waters*

		Patiria sperm	*Pisaster* sperm	*Astropecten* sperm
Patiria egg water +	adjuvant	+++	±	−
	sea water	±	−	−
Pisaster egg water +	adjuvant	−	+++	−
	sea water	−	++	−
Astropecten egg water +	adjuvant	−	−	±
	sea water	−	−	−
	adjuvant + sea water	−	−	−
	Patiria sperm supernatant	−	−	−

It will be seen that *Patiria* and *Pisaster* egg waters agglutinated only homologous sperm. Thus the species specificity rule holds for these two forms. In this experiment *Pisaster* egg water clumped homologous untreated sperm. This reaction did not occur with predictable regularity. The reaction between *Astropecten* egg water and homologous sperm was doubtful. This may be ascribed to neutralization of the *Astropecten* egg water by the *Patiria* sperm supernatant present in the adjuvant solution. The relationship here is somewhat involved. With the exception of the reaction between *Patiria* sperm supernatant and *Astropecten* egg water, the reactions were species specific.

The source of Patiria fertilizin. Only egg water prepared from suspensions of *Patiria* eggs possessing their normal gelatinous coats agglutinated species sperm in the presence of the adjuvant. Blood from female animals did not have this effect. Thus it may be concluded that a specific substance is obtained from starfish eggs which will react with and under certain conditions agglutinate species sperm. This then gives clear and direct support to Tyler's (1941) view that fertilizin exists in the *Patiria* egg water.

THE NATURE OF PATIRIA FERTILIZIN

Tyler (1941) concluded that *Patiria* fertilizin was univalent for combining groups complementary to sperm. It follows from the lattice theory that such univalent fertilizin must become multivalent to agglutinate the sperm. The adjuvant should then convert the natural univalent *Patiria* fertilizin to a multivalent, agglutinating form. However, another possibility in accord with the lattice theory is that the fertilizin is multivalent but the sperm is normally univalent. The results of the following experiments favor this latter view.

Effect of ultraviolet light on Patiria fertilizin. Sea urchin fertilizin can be converted to the univalent form by proper exposure to heat, enzymes, x-radiation and ultraviolet light (Tyler, 1941; Metz, 1942). Such treated fertilizin will not agglutinate sperm but it will combine with sperm rendering the sperm unagglutinable by untreated fertilizin. To test for the possibility of a similar action, *Patiria* fertilizin was exposed to ultraviolet irradiation. It was found that irradiated *Patiria* fertilizin will not agglutinate adjuvant-treated homologous sperm, and normal fertilizin will not subsequently agglutinate the sperm that has been treated with irradiated fertilizin. Thus it is possible that the natural fertilizin is multivalent and the irradiated material is true univalent fertilizin. The data from a typical experiment are given in Table II.

TABLE II

Destruction of agglutinating power of Patiria *fertilizin by ultraviolet light and agglutination inhibiting properties of this fertilizin*

Solution	Irradiated fertilizin	Control fertilizin	Irradiated fertilizin + control fertilizin	Irradiated sea water + control fertilizin
Reaction of adjuvant-treated sperm	−	++++	−	++++

Two stender dishes each containing 5 cc. of a *Patiria* fertilizin solution and one dish containing 5 cc. of sea water were irradiated for 220 minutes. The control fertilizin sample was screened from the ultraviolet light by a "noviol C" filter. After the irradiation the control and irradiated fertilizin samples were tested for agglutinin activity by mixing 2 drops of hen's egg white treated sperm (1%) with 2 drops of each fertilizin solution. At the same time 2 drops each of the sperm and irradiated sea water were mixed. It will be seen that the irradiated fertilizin was inactive whereas the control strongly agglutinated the sperm. After this examination one drop of unirradiated test fertilizin was added to the irradiated fertilizin-adjuvant-sperm mixture and one drop to the irradiated sea water-adjuvant sperm. In this test for inhibition of agglutination it will be seen that sperm treated with irradiated fertilizin did not agglutinate upon subsequent addition of normal fertilizin, whereas the sperm treated with irradiated sea water reacted strongly.

Agglutination of adjuvant-free Patiria sperm by fertilizin. More definite evidence for the multivalent nature of starfish fertilizin was obtained from a study of the effect of adjuvant on sperm. Adjuvant was added to *Patiria* sperm and then removed. Such adjuvant free sperm agglutinated on addition of natural fertilizin. Twenty drops of 0.5 per cent sperm were mixed with 10 drops of isotonic hen's egg white. A control sample consisted of 20 drops of 0.5 per cent sperm plus 10 drops of sea water. Both samples were centrifuged and the packed sperm was resuspended in 20 drops of sea water. Two drops of sperm from each were tested with *Patiria* fertilizin. The control suspension did not react whereas the sperm centrifuged from the egg white agglutinated moderately. The suspensions were recentrifuged and the supernatants tested and found free of adjuvant. The sperm masses were resuspended in 16 drops of sea water after the second centrifugation and tested. The control sperm did not react to fertilizin whereas the sperm previously treated with adjuvant agglutinated weakly.

This experiment was not confirmed with *Astropecten*. *Astropecten* sperm after centrifugation from hen's egg white solution were not agglutinated by homologous fertilizin alone, although this sperm reacted strongly when both egg white and fertilizin were added.

It seems clear then that *Patiria* fertilizin will agglutinate sperm after the adjuvant has been removed from the sperm. It may therefore be concluded that the natural *Patiria* fertilizin is multivalent.

UNIVALENT SPERM

Evidence has just been presented to show that natural *Patiria* fertilizin is multivalent and capable of agglutinating sperm. It follows that the normal sperm is incapable of agglutination. The adjuvant must then convert the sperm to an agglutinating condition.

It seems unlikely that stimulation of the normally immobile sperm to intense activity is of any considerable importance in this adjuvant-fertilizin agglutination of *Patiria* sperm since immunological doctrine does not require motility of cells for agglutination. Thus non-motile bacteria and erythrocytes agglutinate strongly when mixed with specific antibody. Furthermore, heat killed sea urchin sperm agglutinate strongly on addition of fertilizin. However, heat killed *Patiria* sperm did not react when mixed with fertilizin and adjuvant. The deficiency of the normal sperm must then involve the antigenic structure of the cell surface. For agglutination to occur the area of the sperm surface containing groupings complementary to fertilizin must be rather extensive. If this region of the sperm surface were limited in extent and contained but a few or even a single antifertilizin group, the sperm could be considered "univalent" for this particular antigen. Such sperm should not agglutinate when mixed with complementary agglutinin (fertilizin). At best only two or three sperm could clump together. This condition is occasionally observed when untreated *Patiria* sperm and fertilizin are mixed. It has been described as the "granular" reaction.

Absorption of Patiria fertilizin by treated and normal sperm. If normal *Patiria* sperm are "univalent" with respect to exposed antifertilizin groups, the cells must be made multivalent before they can be expected to agglutinate. The adjuvant is believed to effect such a conversion to the multivalent form by "exposing" latent or unreactive antifertilizin present on or near the sperm surface. Treated sperm then should bind more fertilizin than the normal "univalent" sperm. One of three absorption experiments demonstrating this is recorded in Table III.

TABLE III

Absorption of fertilizin by sea water and egg white treated Patiria *sperm*

Absorbing Mixtures

Tube I	Tube II	Tube III	Tube IV
0.5 cc. sea water 0.5 cc. fertilizin 0.5 cc. sperm	0.5 cc. egg white 0.5 cc. fertilizin 0.5 cc. sperm	0.5 cc. egg white 0.5 cc. fertilizin 0.5 cc. sea water	0.5 cc. sea water 0.5 cc. fertilizin 0.5 cc. sea water

Titration of absorbed fertilizin solutions

Dilution of absorption supernatant	Tube I	Tube II	Tube III	Tube IV
1/2	+++	−	++++	++++
1/4	++	−	++++	++++
1/8	+	−	++++	++++
1/16	+	−	+++	+++
1/32	+	−	+++	+++
1/64	+	−	++	++
1/128	+	−	++	++
1/256	−	−	++	++
1/512	−	−	+	+
1/1024	−	−	+	+
1/2048	−	−	−	−

Four absorption tubes were prepared as indicated in the table. Fifty per cent sperm was used in the absorption and raw isotonic hen's egg white was employed as adjuvant. The tubes were refrigerated for nine hours to allow for complete reaction, and then centrifuged. The supernatants were then titrated for fertilizin with one per cent treated sperm. In absorption tubes III and IV sea water was substituted for the sperm added to tubes I and II. No adjustment was made in the titration for the volume of absorbing sperm removed from I and II by centrifugation. This is justified since the titration was made on a comparative basis and tubes III and IV represent controls for neutralization of fertilizin by egg white. Furthermore, the error in absolute values introduced by this involves something less than ⅙ of a dilution and therefore is well within the error of the method. Likewise, no adjustment was made in the supernatant of tube I for the adjuvant present in the absorption supernatant of tube II. Such adjustment was apparently unnecessary since the titers of the control tubes III and IV were the same (1024) The titers of these tubes also show that the egg white does not neutralize fertilizin. Comparison of tubes I and III shows that the sea water-sperm mixture caused an 8- to 64-fold drop in fertilizin concentration. However, in tube II (titer 0) the adjuvant-sperm mixture completely exhausted the fertilizin. The striking difference in the titers of tubes I and II (128 and 0 respectively) demonstrates clearly that treated sperm has a greater fertilizin binding capacity than normal sperm.

Effect of the Adjuvant on the Fertilizing Power of Patiria Sperm

Since the adjuvant increases the fertilizin binding power of sperm and also the motility of these cells, it seemed likely that treated sperm would be unusually effective in fertilization. Several experiments comparing the treated and normal sperm in this respect showed this to be the case. The results of one such experiment are given in Table IV.

Table IV

The effect of hen's egg white on the fertilizing power of Patiria *sperm*

Sperm dilution	Egg white treated sperm	Sea water treated sperm	Egg white + Patiria eggs	Sea water + Patiria eggs
	% cleavage	% cleavage	% cleavage	% cleavage
1/2	94% (75)*	38% (95)*	0.7% (152)*	0.0% (118)*
1/4	95% (66)	0% (58)		
1/8	89% (45)	7.4% (54)		
1/16	88% (50)	6.8% (74)		
1/32	89% (53)	2.0% (65)		

* Total number of eggs counted.

A fresh one per cent sperm suspension was divided into two parts. One part was diluted serially (in twofold steps) with boiled isotonic hen's egg white. The other part was diluted similarly but with sea water. Sperm dilutions are given as the dilution of one per cent sperm added to the eggs. One drop of each sperm suspension was added to twelve drops of *Patiria* eggs in 6 cc. of sea water. To control for parthenogenesis one drop of egg white was added to one dish of eggs and a drop of sea water to a second dish. The eggs were examined for cleavage three hours after addition of sperm.

Although the number of eggs counted was small it can readily be seen that the egg white treatment greatly increased the fertilizing power of the sperm. Even at the lowest dilutions the treated sperm was twice as effective as the untreated cells. At high dilutions the treated sperm fertilized nearly 90 per cent of the eggs whereas the normal sperm fertilized less than 10 per cent. Gray (1915) has reported a similar result with alkali treated *Asterias glacialis* sperm.

Specificity of the Adjuvant

Although no exhaustive search was made for different sources of adjuvant, a number of unrelated preparations were encountered which stimulated *Patiria* sperm and rendered it agglutinable by fertilizin. These preparations included *Panulirus,* rabbit, fish (*Crassius*), and hen sera, and hen's egg white. Thus the source of the adjuvant is not highly specific.

Properties of the Egg White Adjuvant

The adjuvant action can not be attributed to the high pH of raw egg white (Needham, 1931) since the material is active at sea water pH. Therefore, preliminary attempts were made to characterize an "active principle" in the hen's egg white. The agent is quite heat stable. Its activity was retained even after several hours at 100° C. In fact heating increased the activity of the egg white several fold. Ultraviolet light had a similar effect. This suggests the release of inactive bound agent. The "active principle" was quite nondialyzable both before and after heating. It was soluble in saturated ammonium sulfate, but insoluble in strong acetone and alcohol. Thus it is probably neither ordinary protein nor lipoid.

Discussion

From the evidence presented it is concluded that fertilizin is obtained from *Patiria* eggs, and that this fertilizin, although it does not agglutinate normal sperm, is a multivalent agglutinin that reacts with the normal sperm. It is further believed that the exposed antifertilizin of normal *Patiria* sperm is limited to a small area of the sperm surface and contains only a few or even a single combining group complementary to fertilizin. For practical purposes such sperm may be considered "univalent." It is necessary to assume that some antifertilizin is exposed on the normal sperm to explain the absorption of fertilizin by such sperm and to account for the "granular" agglutination reaction. This then is a reversal of Tyler's (1941, 1942) view. He believed that the normal *Patiria* fertilizin was "univalent" and that the sperm was multivalent.

The various adjuvant solutions stimulate the sperm to intense motility and presumably expose more antifertilizin on the sperm surface. The latter effect is believed to be the essential one in rendering the sperm agglutinable. This action of the adjuvants bears a superficial resemblance to the "transformation" of human erythrocytes by an enzyme present in certain bacterial filtrates (Thomsen effect). Any human serum will agglutinate these transformed cells. There are several important differences between the process of erythrocyte transformation (Friedenrich, 1930) and the action on starfish sperm. The transformation requires a considerable period of time (15 minutes to several hours), is irreversible, and involves a fixation and subsequent release of the transforming principle. The action on *Patiria* sperm takes place very rapidly, the process reverses slowly when the adjuvant is removed, and it involves no fixation of the adjuvant. Repeated attempts failed to show any neutralization or absorption of egg white adjuvant by sperm or sperm-fertilizin mixtures. Friedenrich (1930) believes that a new agglutinogen is developed which is not present in latent or unreactive form on the normal erythrocyte. However, the case of *Patiria* sperm is more easily explained by assuming that a considerable amount of antifertilizin is in latent form on or near the cell surface.

Di Macco's (1923) "coagglutination" of sheep erythrocytes by mixtures of ricin and guinea pig serum also resembles the fertilizin-adjuvant agglutination of *Patiria* sperm. Neither ricin nor guinea pig serum alone agglutinated the sheep cells. Absorption of the separate solutions with cells failed to remove the active agents. Agglutination failed to occur if the ricin and guinea pig serum were mixed first and the sheep cells added subsequently. Thus neither of the necessary agents reacted directly with the cells. Di Macco concluded that agglutination of sheep cells resulted from a reaction between the cells and an evanescent ricin-serum complex formed at a critical stage in the reaction between these substances. It is apparent, then, that the mechanism of the coagglutination is fundamentally different from the fertilizin-adjuvant agglutination of *Patiria* sperm.

The striking difference in fertilizing power of normal and adjuvant-treated sperm can be explained by the motility of the cells. Furthermore, this effect should be expected, regardless of motility, from the recent views of Tyler (1941). He has shown that fertilizin treatment lowers the fertilizing power of *Patiria* sperm and explained this by assuming that at fertilization a union occurs between antifertilizin on the sperm and fertilizin at the egg surface. If all of the sperm antifertilizin is bound by free fertilizin, then no reaction can occur between sperm and the surface of the egg. It follows from this that the normal univalent sperm would have much less chance of reaching the egg surface in an unsaturated condition than would the multivalent sperm. At present it is impossible to judge the relative importance of the intense motility and the multivalency of the adjuvant-treated sperm in this fertilization effect. If this increased fertilizing power should be found in species that regularly give low percentages of fertilized eggs, it might be useful for technical purposes.

SUMMARY

I. Starfish sperm does not ordinarily agglutinate when treated with homologous fertilizin. However, when the sperm of some species (*Patiria miniata, Pisaster ochraceus, Astropecten* sp.) is treated with certain adjuvants the cells become intensely active and agglutinate when fertilizin is added. This reaction provides a means for studying the relationship between starfish sperm and fertilizin.

II. *Patiria* sperm will combine with homologous fertilizin and remove it from solution even in the absence of the adjuvant.

III. Cross tests between *Patiria, Pisaster* and *Astropecten* sperm and fertilizin solutions revealed no cross agglutination reactions.

IV. It is concluded that *Patiria* fertilizin is multivalent, since irradiated fertilizin will not agglutinate treated sperm but will inhibit the agglutination of such sperm by normal fertilizin; and since normal fertilizin will agglutinate sperm which has been freed of adjuvant.

V. It is suggested that normal *Patiria* sperm possesses but a single antifertilizin combining group and that more such groups are exposed on the sperm surface through the action of the adjuvant. Experiments which show that the fertilizin binding power of sperm is increased by the adjuvant support this view.

I am most grateful to Dr. Albert Tyler for the aid and encouragement he has given throughout the course of the work.

LITERATURE CITED

Di Macco, G., 1923. Ueber die coagglutinierende und präzipitierende Wirkung des Rizins. *Zeitschr. f. Immunit.*, **38**: 467–488.

Frank, J. A., 1939. Some properties of sperm extracts and their relationship to the fertilization reaction in Arbacia punctulata. *Biol. Bull.*, **76**: 190–216.

Friedenreich, V., 1930. *The Thomsen Hemagglutination phenomenon.* Levin and Munksgaard, Copenhagen.

Glaser, O., 1914. A quantitative analysis of the egg secretions and extracts of Arbacia and Asterias. *Biol. Bull.,* **26**: 367–386.

Gray, J., 1915. Notes on the relation of spermatozoa to electrolytes and its bearing on the problem of fertilization. *Quart. Jour. Microscopical Science, N.S.,* **61**: 119–126.

Heidelberger, M., 1938. *Chemistry of amino acids and proteins,* Chap. XVII. C. C. Thomas, Springfield, Ill.

Just, E. E., 1930. The present status of the fertilizin theory of fertilization. *Protoplasma,* **10**: 300–342.

Lillie, F. R., 1913. Studies of fertilization. V. The behavior of the spermatozoa of Nereis and Arbacia with special reference to egg extractives. *Jour. Exp. Zool.,* **14**: 515–574.

Lillie, F. R., 1919. *Problems of fertilization.* University of Chicago Press, Chicago.

Loeb, J., 1914. Cluster formation of spermatozoa caused by specific substances from eggs. *Jour. Exp. Zool.,* **17**: 123–140.

Marrack, J. R., 1938. *The chemistry of antigens and antibodies.* Medical Research Council, Special Report Series, No. 230, London.

Metz, C. B., 1942. The inactivation of fertilizin and its conversion to the "univalent" form by x-rays and ultraviolet light. *Biol. Bull.,* **82**: 446–454.

Metz, C. B., 1944. Agglutination of starfish sperm by homologous egg water. *Anat. Rec.,* **89**: 559.

Needham, J., 1931. *Chemical embryology,* Vol. I. Macmillan Co., New York.

Tyler, A., 1939. Crystalline echinochrome and spinochrome: their failure to stimulate the respiration of eggs and sperm of Strongylocentrotus. *Proc. Nat. Acad. Sci.,* **25**: 523–528.

Tyler, A., 1940. Sperm agglutination in the keyhole limpet Megathura crenulata. *Biol. Bull.,* **78**: 159–178.

Tyler, A., 1941. The role of fertilizin in the fertilization of eggs of the sea urchin and other animals. *Biol. Bull.,* **81**: 190–204.

Tyler, A., 1942. Specific interacting substances of eggs and sperm. *Western Jour. Surgery, Obstetrics and Gynecology,* **50**: 126–138.

Tyler, A., and C. B. Metz, 1944. Natural heteroagglutinins in lobster serum. *Anat. Rec.,* **89**: 568.

Woodward, A. E., 1918. Studies on the physiological significance of certain precipitates from the egg secretions of Arbacia and Asterias. *Jour. Exp. Zool.,* **26**: 459–497.

COCHLIOPHILUS DEPRESSUS GEN. NOV., SP. NOV. AND COCHLIOPHILUS MINOR SP. NOV., HOLOTRICHOUS CILIATES FROM THE MANTLE CAVITY OF PHYTIA SETIFER (COOPER)

EUGENE N. KOZLOFF

Department of Zoology, University of California

Introduction

Examination of specimens of the pulmonate snail *Phytia setifer* (Cooper) [1] from salt marshes bordering San Francisco Bay disclosed the presence of two closely related species of flattened holotrichous ciliates within the mantle cavity. A new genus, *Cochliophilus,* is proposed to include these ciliates, which will be described herein as *Cochliophilus depressus* gen. nov., sp. nov. and *Cochliophilus minor* sp. nov.

I wish to express my appreciation to Professor S. F. Light and Professor Harold Kirby for their interest and helpful advice during the progress of this investigation.

Technique

Phytia setifer occurs under matted vegetation and debris in salt marshes and in the vicinity of brackish water ponds on the Pacific Coast of central and northern California. Material for this study was collected at several localities along the east shore of San Francisco Bay at Oakland and Berkeley.

For observation of the living ciliates the shell of the snail was carefully removed and the anterior part of the animal crushed in a drop of sea water on a slide. Fixation of the organisms for permanent preparations was accomplished by liberating them in this manner on a coverglass and then dropping the coverglass smear-side down onto the surface of the fixative in a Petri dish.

Staining with iron hematoxylin gave good results following the fixatives of Schaudinn, Champy, Bouin, and Heidenhain ("susa"). For a study of the ciliary system the method devised by Bodian (1936, 1937) for impregnation with activated silver albumose (protargol) was used after fixation in Hollande's cupric-picro-formol mixture. The Feulgen nuclear reaction was tried with success on material fixed in a saturated aqueous solution of mercuric chloride with 5 per cent of glacial acetic acid.

Description of Species

There is no agreement among protozoologists in regard to the orientation for descriptive purposes of compressed ciliates in which the cytostome is situated along the margin of the flattened body or displaced to the surface opposite that in contact with the substrate. Hentschel (1924), writing of *Entodiscus* (*Cryptochilum*) *borealis,* stated that "since convention dictates that the side on which the mouth is situated shall be called ventral, we must say that the animal is flattened from side to side." Reichenow (1927–29) applied this scheme to *Conchophthirus,* as did also Kahl (1931, 1934) and Raabe (1932, 1934b).

---

[1] Dall (1921) has implied that the species described by Cooper (1872) is distinct from *Phytia myosotis* (Drap.) of Europe and the Atlantic Coast of North America. No conclusive evidence has been presented to support or to refute this contention.

De Morgan (1925), in his description of *Kidderia* (*Conchophthirus*) *mytili,* considered the concave under-surface to be ventral and the position of the cytostome to be lateral. Kidder (1933b) recognized the oral surface of *Kidderia mytili* as the "physiological ventral surface," but for purposes of clearness accepted De Morgan's plan of orientation. In the present paper I will follow De Morgan and Kidder in referring to that surface of the body most often found in contact with the substrate as ventral. The lateral margin on which the cytostome is situated will be referred to as the oral margin, and the opposite side as the aboral margin.

Cochliophilus depressus gen. nov., sp. nov. (*Figs. 1 and 2*)

The body outline as seen from the dorsal or ventral aspect is ovoid, often somewhat truncate at the posterior end. A view from the oral or aboral margin shows this ciliate to be much flattened, the ventral surface being slightly concave and the dorsal surface convex. In some individuals the curvature of the dorsal surface appears to be less regular than in others.

Twenty living individuals taken at random ranged from 70 μ to 107 μ in length and from 47 μ to 77 μ in width, averaging about 93 μ by 63 μ. The thickness varied from 11 μ to 16 μ. The relation of the length to the width is not the same in all specimens. Fixation of the organisms on coverglasses produced some shrinkage and frequently also distortion of shape due to compression.

The elongated peristomal area is situated in the posterior fourth of the body. Specialized ciliary elements which will be described presently extend from the anterior end of the peristomal indentation to the cytostome. That part of the peristomal area lying posterior to the cytostome is naked.

A well-defined pharynx is not present. I prefer to regard the irregular tubular structure which passes from the cytostome into the cytoplasm as the gullet. This gullet is difficult to see in living individuals, but in fixed material is demonstrable following staining in iron hematoxylin. As it approaches the macronucleus the gullet widens out and its boundaries become inexact.

A thin pellicle covers the body. Flexure of the pellicle in this ciliate is rarely noted, and then only when the animal comes in contact with solid obstructions in its path of movement. Trichocysts are absent.

The cilia of the body are disposed in 52 to 56 longitudinal rows and beat metachronously. The cilia on the dorsal and ventral surfaces are somewhat longer than those along the margin. The ventral cilia are thigmotactic, but not strongly so. On the ventral surface at the anterior end is a transverse suture (anterior field) from which the ventral rows of cilia extend backward, and from which the dorsal rows curve upward and continue posteriorly. Most of the dorsal rows converge in a characteristic pattern towards the posterior end. A definite unciliated area is evident between the longer dorsal rows and the ventral rows which curve upward a short distance over the posterior end.

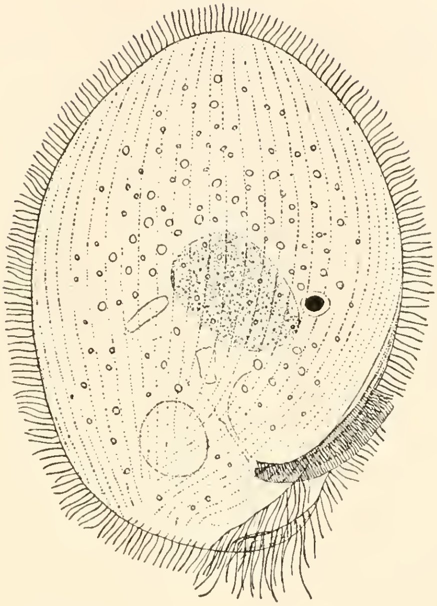


FIGURE 1. *Cochliophilus depressus* gen. nov., sp. nov. Dorsal aspect. Heidenhain's fixative ("susa")-iron hematoxylin. Drawn with aid of camera lucida. × 900.

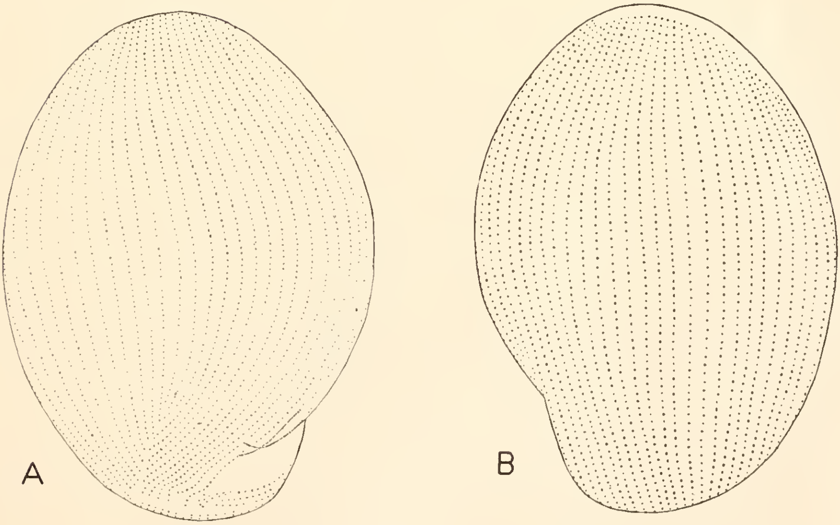


FIGURE 2. *Cochliophilus depressus* gen. nov., sp. nov. Distribution of ciliary rows. Hollande's fixative-protargol. Drawn with aid of camera lucida. *A*. Dorsal aspect. Though distorted somewhat due to compression, this individual shows well the arrangement of ciliary rows entering the peristomal indentation. × 670. *B*. Ventral aspect. × 950.

Three ventral rows of cilia close to the oral margin turn dorsally near the end of their course to delimit the naked part of the peristomal area posteriorly. The first of these rows is ordinarily seen to ramify into an incomplete double or triple series of cilia. The post-peristomal extensions of the ventral rows and the terminal part of the dorsal row which borders the peristome above bear cilia which are two to three times as long as the peripheral cilia elsewhere on the body.

The specialized peristomal cilia arise from two series of closely-set basal granules, each of which is seen to be a continuation of two rows of peripheral cilia essentially lateral in position, lying between the three ventral rows of cilia and one dorsal row marking off the peristomal area. The cilia of the upper peristomal row are appreciably longer than those of the lower row and appear in living individuals to form a membrane-like structure which beats up and down as a unit. The cilia of the lower row are much thicker and do not beat synchronously. The activity of the peristomal cilia ceases soon after the organism is dissociated from the host.

The cytoplasm is colorless. Greenish granules appearing as highly refractile bodies are distributed through the cytoplasm. These are most numerous around the macronucleus and following fixation stain intensely with iron hematoxylin.

The macronucleus is centrally located. In outline it varies from oblong to round, and in life is conspicuous as a clear granular body surrounded by food inclusions and cytoplasmic granules. The micronucleus is greenish in color and difficult to detect in living individuals. It is easily demonstrated by iron hematoxylin or the Feulgen nuclear reaction. The micronucleus is commonly situated close to the macronucleus, between the latter and the oral margin. Upon fixation it shrinks considerably and draws away from the membrane by which it is invested.

The contractile vacuole lies in the posterior fourth of the body behind the gullet, and apparently opens to the exterior at a point between the convergence of the shorter dorsal ciliary rows. I have been unable to distinguish a permanent opening in the pellicle.

When free in water, *Cochliophilus depressus* swims actively, generally in circles and with its concave ventral surface in contact with the substrate. Occasionally, however, it follows an erratic course, rotating on its longitudinal axis. The transverse anterior field is always at right angles to the direction of movement. In the presence of pieces of tissue from the host *Cochliophilus depressus* will sometimes seek refuge among them or cling to epithelial surfaces by means of its ventral thigmotactic cilia.

I have found *Cochliophilus depressus* to be present in the mantle cavity of nearly all specimens of *Phytia setifer* which I have examined. It occurs in small numbers and is usually less common than the following species.

Cochliophilus minor sp. nov. (*Figs. 3 and 4*)

The shape of this species resembles in general that of *Cochliophilus depressus,* except that the posterior end is rather pointed, never truncate, and the dorsoventral dimension in relation to the length and breadth is comparatively greater. In addition, the curvatures of the ventral and dorsal surfaces are more pronounced in *Cochliophilus minor.*

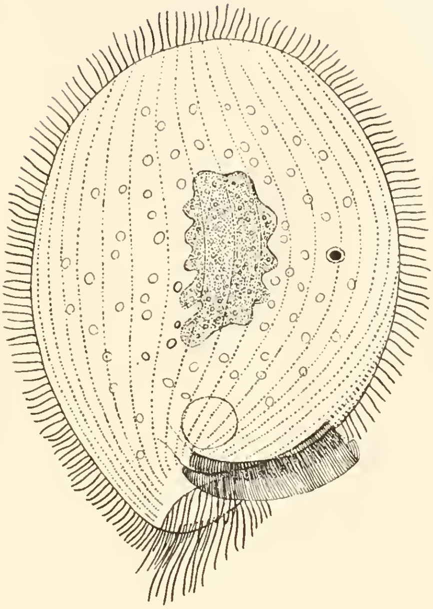


Figure 3. *Cochliophilus minor* sp. nov. Dorsal aspect. Heidenhain's fixative ("susa")-iron hematoxylin. Drawn with aid of camera lucida. × 1250.

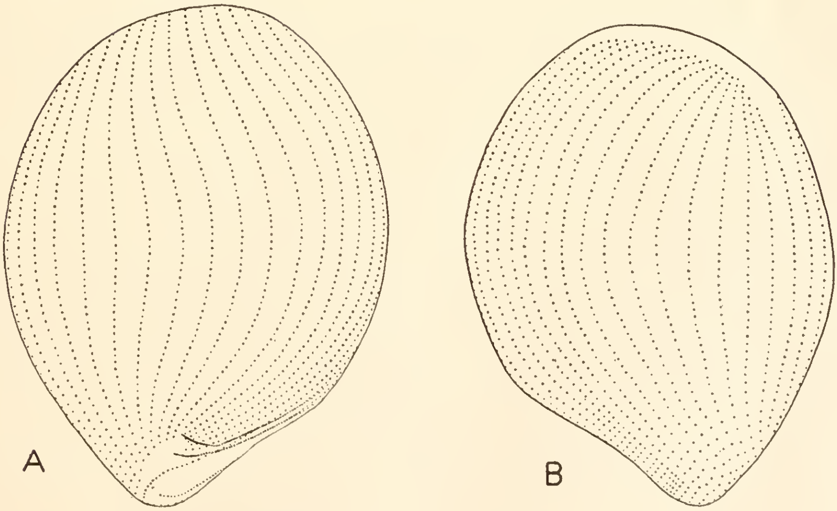


Figure 4. *Cochliophilus minor* sp. nov. Distribution of ciliary rows. Hollande's fixative-protargol. Drawn with aid of camera lucida. *A*. Dorsal aspect. × 1250. *B*. Ventral aspect. × 1250.

Twenty living individuals taken at random ranged from 51 μ to 80 μ in length and from 33 μ to 56 μ in width, averaging about 63 μ by 45 μ. The thickness varied from 11 μ to 18 μ.

The peristomal area is situated in the posterior fourth of the body. Two rows of specialized cilia extend from the anterior end of the peristomal indentation to the cytostome. That part of the peristomal area posterior to the cytostome is naked.

An irregular gullet may sometimes be traced a short distance from the cytostome, but it is not as easily discerned as the comparable structure in *Cochliophilus depressus.*

The cilia are disposed in 36 to 38 longitudinal rows and beat metachronously. The cilia on the ventral and dorsal curvatures are slightly longer than those along the margin. The ventral cilia are weakly thigmotactic. The ventral rows extend from an anterior transverse suture to the posterior tip of the body. The basal granules of most of the ventral rows come to lie farther apart towards the posterior end, while those of three or four rows near the oral margin lie closer together. The dorsal rows of cilia pass from the transverse suture over the anterior end of the body and continue backward to terminate in a conformation homologous with that found in *Cochliophilus depressus.* The posterior dorsal unciliated area of *C. depressus* has no exact homologue in this species. There exists, nevertheless, an unciliated area between the converging dorsal rows and the dorsal row bordering the peristomal area above.

One or two rows of cilia following the oral margin curve dorsally near the end of their course to delimit the naked part of the peristomal area posteriorly. These extensions and the terminal part of the most nearly lateral dorsal row on the oral side bear exceptionally long cilia.

The peristomal ciliary apparatus consists of a membrane-like structure of long, fine cilia which curves downward over a row of closely-set, rather thick cilia extending from the anterior end of the peristomal indentation to the cytostome. The membrane-like structure appears to be non-motile and to function as a funnel directing food particles into the cytostome.

The cytoplasm is colorless. Refringent cytoplasmic granules are present, but to a lesser extent than in *Cochliophilus depressus.*

The size and shape of the macronucleus are highly variable. In living as well as fixed individuals it is nearly always seen to be ramified, although ovoid or round macronuclei are occasionally noted in this species. Reorganization stages in which two or more smaller and round macronuclei are present are not infrequently met with. The micronucleus ordinarily occupies a position between the macronucleus and the oral margin. In fixed and stained preparations it is considerably shrunken.

The contractile vacuole is situated anterior to the cytostome. It opens to the exterior between the convergence of the shorter dorsal ciliary rows. I have not detected a permanent opening in the pellicle.

When separated from its host *Cochliophilus minor* swims in circles or proceeds forward rotating on its longitudinal axis. Its movements are in general slower than those of *Cochliophilus depressus.*

Cochliophilus minor is found in association with *Cochliophilus depressus* in the mantle cavity of *Phytia setifer.* It is usually more numerous than *C. depressus.*

Systematic Position

On the basis of certain features of the morphology of the two species of *Cochliophilus* which I have described it may be justifiable to allocate this genus to the sub-order *Thigmotricha* Chatton and Lwoff, although in view of the deficiencies of the systems of classification of holotrichous ciliates currently recognized I must defer a conclusive statement with regard to its position. The organization of the peristome of *Cochliophilus* hints its affinity with *Kidderia* Raabe, represented by *K. mytili* (De Morgan) from *Mytilus edulis*. Raabe (1936) retained *Kidderia* in the family Conchophthiridae Reichenow,[2] but removed to the family Thigmophryidae Chatton and Lwoff *Myxophyllum* and *Conchophyllum,* genera created by him to accommodate, respectively, Stein's species *Conchophthirus steenstrupi,* commensal on various terrestrial pulmonate molluscs, and *Conchophthirus caryoclada* Kidder, from the bivalve *Siliqua patula*. It is interesting to note, in passing, that a specific character of *Conchophyllum caryoclada* is its branched macronucleus, of which the macronucleus of *Cochliophilus minor* is reminiscent.

The presence of a membrane-like structure in the peristome of *Cochliophilus* could be the basis for objections to the inclusion of this genus in the Thigmotricha. Very similar ciliary elements have been observed, however, in certain species of the family Ancistrumidae Issel. Raabe (1932, 1934b) has stressed the presence of an undulating membrane in *Conchophthirus*, although Kidder (1934), after studying species of *Conchophthirus* from fresh water mussels in this country, was unable to corroborate Raabe's findings, and suggested that Raabe may have mistaken the fibers of the peristomal basket for an undulating membrane.

Genus Cochliophilus gen. nov.

Diagnosis: Flattened holotrichous ciliates, ovoid in outline as seen in dorsal or ventral view. The peristomal area is elongated and is situated on the right lateral margin in the posterior fourth of the body. A membrane-like structure of fine cilia overlies a series of thick cilia extending from the anterior end of the peristomal indentation to the cytostome; that part of the peristomal area posterior to the cytostome is naked. The peripheral cilia are disposed in longitudinal rows extending from a ventral transverse suture at the anterior end of the body. The dorsal rows converge in a characteristic pattern posteriorly. Thichocysts are absent. The macronucleus is centrally located; the micronucleus is usually situated near the macronucleus, between the latter and the oral margin. The contractile vacuole opens to the exterior between the convergence of the shorter dorsal ciliary rows; no permanent opening in the pellicle is discernible. Genotype: *Cochliophilus depressus* gen. nov., sp. nov. Two species, commensal in the mantle cavity of *Phytia setifer* (Cooper).

Cochliophilus depressus gen. nov., sp. nov.

Diagnosis: Average size about 93 μ by 63 μ, the thickness being about one-sixth the length. The ciliary rows are 52 to 56 in number. The peristomal membrane-like structure is motile. The macronucleus is round or oblong. Syntypes are in the collection of the author.

---

[2] Reichenow (1927–29) was apparently the first to use the name Conchophthiridae, although Raabe credits Kahl (1931) with establishing this family.

Cochliophilus minor sp. nov.

Diagnosis: Average size about 63 μ by 45 μ, the thickness being about one-fourth the length. The ciliary rows are 36 to 38 in number. The peristomal membrane-like structure is apparently immobile, serving as a funnel directing food particles into the cytostome. The macronucleus is characteristically ramified. Syntypes are in the collection of the author.

LITERATURE CITED

Bodian, D., 1936. A new method for staining nerve fibers and nerve endings in paraffin sections. *Anat. Rec.,* **69**: 89.

Bodian, D., 1937. The staining of paraffin sections of nervous tissue with activated protargol. The role of fixatives. *Anat. Rec.,* **69**: 153.

Cooper, J., 1872. On new Californian Pulmonata, etc. *Proc. Acad. Nat. Sci. Philadelphia,* **24**: 143.

Dall, W., 1921. Summary of the marine shellbearing mollusks of the northwest coast of North America, from San Diego, California, to the Polar Sea. *U. S. Nat. Mus. Bull.* 112.

De Morgan, W., 1925. Some marine ciliates living in the laboratory tanks at Plymouth, with a description of a new species, Holophrya coronata. *Jour. Mar. Biol. Assoc. United Kingdom,* **13** (n.s.) : 600.

Hentschel, C., 1924. On a new ciliate, Cryptochilum boreale, sp. nov., from the intestine of Echinus esculentus Linn., together with some notes on the ciliates of echinoids. *Parasitology,* **16**: 321.

Kahl, A., 1931. Urtiere oder Protozoa. I: Wimpertiere oder Ciliata (Infusoria). 2. Holotricha. In Dahl, F.: Die Tierwelt Deutschlands, 21 Teil. Gustav Fischer, Jena.

Kahl, A., 1934. Ciliata entocommensalia et parasitica. In Grimpe, G., and E. Wagler: Die Tierwelt der Nord- und Ostsee, Lief. 26 Teil II C_1. Akademische Verlagsgesellschaft, Leipzig.

Kidder, G., 1933a. Conchophthirius caryoclada sp. nov. (Protozoa, Ciliata). *Biol Bull.,* **65**: 175.

Kidder, G., 1933b. Studies on Conchophthirius mytili De Morgan. I. Morphology and division. *Arch. Protistenk.,* **79**: 1.

Kidder, G., 1934. Studies on the ciliates from fresh water mussels. I. The structure and neuromotor system of Conchophthirius anodontae Stein, C. curtus Engl., and C. magna sp. nov. *Biol. Bull.,* **66**: 69.

Raabe, Z., 1932. Untersuchungen an einigen Arten des Genus Conchophthirus Stein. *Bull. int. Acad. Cracovie, Cl. Sci. math. nat.,* B (II), **1932**: 295.

Raabe, Z., 1934a. Über einige an den Kiemen von Mytilus edulis L. und Macoma balthica (L.) parasitierende Ciliaten-Arten. *Ann. Mus. zool. polon.,* **10**: 289.

Raabe, Z., 1934b. Weitere Untersuchungen an einigen Arten des Genus Conchophthirus Stein. *Mém. Acad. Cracovie, Cl. Sci. math. nat.,* B (II), **1934**: 221.

Raabe, Z., 1936. Weitere Untersuchungen an parasitische Ciliaten aus dem polnischen Teil der Ostsee. I. Ciliata Thigmotricha aus den Familien: Thigmorphryidae, Conchophthiridae und Ancistrumidae. *Ann. Mus. zool. polon.,* **11**: 419.

Reichenow, E., 1927–29. Lehrbuch der Protozoenkunde. 5th ed. Gustav Fischer, Jena.

THE DEVELOPMENT OF MARINE FOULING COMMUNITIES

BRADLEY T. SCHEER

Wm. G. Kerckhoff Marine Laboratory, California Institute of Technology, Corona del Mar

This paper constitutes an examination of the sedentary communities found on float bottoms and other submerged objects in Newport Harbor, California. Particular attention has been paid to the changes in composition of such communities with time.

The basic problem in the development of a sequence of communities in a limited environment is that of distinguishing between seasonal progression and true succession. Seasonal progression results fundamentally from differences in breeding seasons of various organisms. This type of development was noted at Beaufort, N. C. by McDougall (1943). Most of the organisms observed by McDougall had short life cycles and short breeding seasons. As a result, most of the organisms which settled in the winter months were dead or moribund by spring, and were replaced by organisms breeding in the latter season.

Succession, in contrast to seasonal progression, involves definite relations between organisms, Shelford (1930) has suggested the following criteria for the occurrence of succession: (1) Early forms must drop out, and be replaced by later forms, and (2) Some of the earlier forms must be essential for the establishment of the later forms. The use of the word "essential" in this connection is perhaps unfortunate. It would be nearly impossible, in most cases, to prove that one organism is essential for the establishment of another. On the other hand, the presence of one organism might well provide conditions favoring the establishment of another, and certainly such favorable conditions would suffice to insure the displacement of early settlers by later arrivals.

The phenomena of ecological succession are well known in terrestrial communities. In littoral marine communities, it has sometimes been stated that true succession does not occur, or is of little importance (Shelford, 1930; McDougall, 1943). The clearest case of succession in intertidal communities is that reported by Hewatt (1935). In the *Mytilus californianus* community characteristic of exposed rocky coasts along the entire Pacific coast of the United States, the establishment of a climactic condition requires more than two and one-half years, and involves a definite sequence of organisms. The reports of Kitching (1937), Moore (1939) and Moore and Sproston (1940) also give some indication that recolonization of intertidal rock surfaces is a slow process. It appears that the first event is ordinarily a heavy settlement of algae, and that many animal forms appear only after the plants have become established. Kitching (1937) provides evidence of a succession of algal forms on rocky intertidal ledges.

The sedentary organisms inhabiting floats, pilings, boat bottoms and similar structures have been the subject of many investigations. The literature in this field has been reviewed recently by McDougall (1943) and need not be cited extensively here. The most thorough investigations dealing with the Pacific forms are those of Coe (1932) and Coe and Allen (1937). These studies, covering a period of nine years, have provided invaluable information regarding the biology of the organisms concerned. The data reported in the current study have been accumulated between February 1943 and March 1945.

THE FLOAT-BOTTOM COMMUNITIES OF NEWPORT HARBOR

Field observations on float bottoms and similar structures in Newport Harbor disclosed the existence of five or six rather definite communities. For convenience, throughout this paper, these communities will be referred to by designations indicating the most abundant organisms in the community. In this way, we may designate (a) algal, (b) bryozoan, (c) *Ciona,* (d) *Styela,* (e) *Mytilus,* and (f) *Balanus* communities. These communities were not all sharply marked off, one from another, and communities intermediate in composition between algal and bryozoan, bryozoan and *Styela, Styela* and *Mytilus,* bryozoan and *Mytilus,* and *Ciona* and *Mytilus* have been observed. The various communities showed no relation to the position of the floats in the harbor, and indeed several different communities were found within a distance of a hundred feet on different floats. Evidence will be presented that this results from a definite succession, and that the composition of the community on any particular float bottom depends on (a) the length of time during which the float has been in the water, and in part on (b) the season during which the float was first immersed. We shall first consider the composition of the various communities.

The bottoms of floats were examined with the aid of a periscopic device involving an ordinary underwater viewing glass with a mirror attached (Fig. 1). Organisms were also removed from floats with a long-handled scraper.

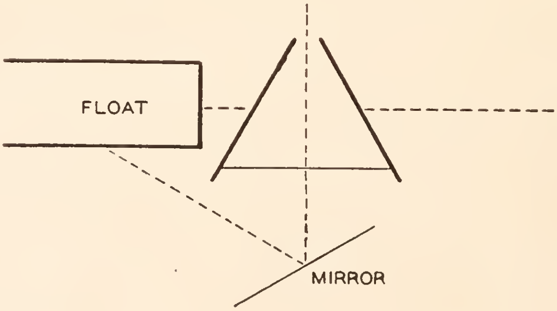


FIGURE 1. Apparatus for the examination of float bottoms.

The algal community. When a clean surface was placed in the bay, the first settlers were bacteria, algae, protozoans, and, during the cooler months of the year, hydroids. The algae included small sedentary diatoms which have not been identified in the present study (see Coe, 1932; and Coe and Allen, 1937), colonial diatoms of the genus *Licmophora,* and one or more species of *Ectocarpus,* notably *E. granulosoides.* In addition, *Enteromorpha* sp., *Lophosiphonia villum,* and *Pterosiphonia bipinnata* were frequently noted. The sedentary protozoans included a form similar to *Zoothamnium,* and the suctorian *Ephelota.* There were seven or eight species of hydroids; these were not identified, but *Obelia dichotoma* was usually conspicuous. Bryozoans were found in this community, sometimes in abundance. On float bottoms, *Bugula neritina* may be an important member of the community, and *Membranipora tuberculata* was observed in one instance on glass plates. *Eucratea clavata,* a small semi-erect bryozoan, occasionally occurred in considerable numbers on glass plates. Finally, young colonies of a number of other species of bryozoans appeared after a time. These will be discussed in more detail later.

The bryozoan community. A good many floats supported a very heavy growth of bryozoans. The principal organisms involved were the encrusting bryozoans *Schizoporella unicornis, Cryptosula pallasiana, Rhynchozoon tumulosum* and *Holoporella aperta.* The erect bryozoans were less constant in occurrence, but were quite abundant in some cases. *Bugula neritina* was less frequent in this community than among the algae, while *Eucratea clavata* was more frequently found among the encrusting bryozoans than among the algae. *Crisulipora occidentalis* and *Scrupocellaria diegensis* were usually present and often very abundant among the bryozoans. Four or five other species of erect bryozoans occurred less frequently.

Although the bryozoans by far outnumbered the other members of this community in most cases (Table VII), other organisms were often quite abundant. Notable are the serpulid worm *Eupomatus gracilis,* and the colonial amphipod *Erichthonius braziliensis. Eupomatus* was almost always found, with its winding calcareuos tubes, between the colonies of encrusting bryozoans. Occasionally, it was very abundant, the tubes making a more or less solid mass. *Erichthonius* was irregular in occurrence. During 1943, it did not appear in quantity, but in 1944 it was extremely abundant during July and August, the mud tubes often covering as much as half of the area of a glass plate. Coe and Allen (1937) noted a similar variation at La Jolla. The ascidians *Styela barnharti, Halocynthia johnsoni,* and *Ciona intestinalis,* and the mussel *Mytilus* sp. were found among the bryozoans in many cases, but since they were more characteristic of other communities, they will be dealt with later. Many crustaceans, annelids and other motile forms used the bryozoan clumps for shelter.

The Ciona community. The previous paragraphs have dealt with communities in which several species were abundant and the proportions of each species showed considerable variation in different communities of the same type. Most of the *Ciona* communities, in contrast, were composed almost wholly of specimens of *Ciona intestinalis.* This was particularly true during the summer and fall, when these communities were at their peak of development. Many float bottoms presented a solid mass of *Ciona,* with only a few other organisms present. These latter were usually colonial ascidians, growing on the tests of the *Ciona,* and such crustaceans and annelids as might have taken refuge among the stalks.

The Styela community. This was a poorly defined community, intermediate in composition between the bryozoan and *Mytilus* communities. The encrusting bryozoans noted earlier were usually present, forming a substratum for the stalks of *Styela,* while the erect bryozoans were often found among these stalks. Small specimens of *Mytilus* were often attached to the stalks in large numbers. Large sponges, which have not been identified, were also frequently present, sometimes in such quantity as to dominate the community. It might indeed be preferable to refer to a *Styela*-Sponge community.

The Mytilus community. *Mytilus* was without question the most abundant dominant on the float bottoms in Newport Bay during the period of this study. This has not always been the case, according to reliable observers (G. E. MacGinitie, A. M. Strong, personal communications); during several previous years, *Mytilus* has not been abundant in the bay. The exact identity of this mussel remains in doubt. It is probably the same form which has been recorded infrequently from this area as *M. edulis.* However, conchologists are not entirely agreed that this is the proper designation. It is certainly not *M. californianus.* The *Mytilus* communities sometimes were observed on a substratum of old and badly decayed bryozoans; at other times they were attached directly to the float bottom. Old specimens of *Styela* or *Ciona* were often present among the mussel clumps, and various types of sponge were often quite abundant.

The Balanus community. Communities in which *Balanus* is the dominant organism were not observed on float bottoms in Newport Harbor, although they are frequently observed on experimental surfaces exposed in the open sea at La Jolla. Indeed, *Balanus tintinnabulum* is probably the principal dominant at La Jolla (Coe, 1932). One experimental panel exposed at this laboratory developed a *Balanus* community comparable to those observed at La Jolla, however.

Changes in Float-Bottom Communities

Eight floats, all located along the mainland side of the channel between Balboa Island and Corona del Mar, and within a distance of 100 yards of one another, were selected in September of 1944, and kept under observation for a period of six months. The results of this study are presented in Table I. At intervals of about one month, the bottom of each float was examined with the viewing glass, and samples of the population removed by hand and with the scraper for later examination in the laboratory.

Float number one had been immersed in the bay for only about one week previous to the first examination. It had at that time (Sept. 21) a typical algal community, with a few specimens of *Bugula.* In October, examination showed increased numbers of *Bugula,* and a few small colonies of other erect bryozoans. In November, *Bugula* and the encrusting bryozoan *Holoporella* had displaced the algae, and a number of small specimens of *Ciona* were present. The float was then covered with a typical bryozoan community. During the remainder of the period, until March, the encrusting bryozoans continued to increase in numbers and size.

Floats 2 and 3 supported typical bryozoan communities in September. In addition to the bryozoans, a number of specimens of *Ciona* were observed, and several small *Mytilus.* During the period of observation, *Mytilus* grew at the expense of the bryozoans and ascidians, becoming very abundant in December, and largely dominating the community by February. The two float populations were very similar in composition in September, but the presence of *Styela* on float 3 in October appears to have favored the earlier establishment of *Mytilus* on this float. The presence of sponges on this float may also be related to *Styela.* Float 4, in Sep-

TABLE I

Changes in composition of float-bottom populations, September 1944 to March 1945

Float number		September 21, 1944	October 23, 1944	November 22, 1944	December 21, 1944	February 13, 1945	March 26, 1945
1	Dominants	Algae	Algae	Bugula Holoporella	Bugula Holoporella	Encrusting Bryozoans	Encrusting Bryozoans
	Sub-dominants		Bugula	Ciona	Ciona		
	Influents	Bugula	Erect Bryozoans Ciona	Encrusting Bryozoans Eupomatus	Encrusting Bryozoans Eupomatus	Scrupocellaria Hydroids	Scrupocellaria Hydroids
2	Dominants	Encrusting Bryozoans Eupomatus	Encrusting Bryozoans Eupomatus	Encrusting Bryozoans Eupomatus	Mytilus Encrusting Bryozoans	Mytilus Encrusting Bryozoans	Mytilus Encrusting Bryozoans
	Sub-dominants	Ciona Bugula Scrupocellaria	Ciona Bugula Scrupocellaria	Ciona Mytilus	Ciona Erect Bryozoans	Erect Bryozoans	Erect Bryozoans
	Influents	Colonial Ascidians Mytilus Sponges	Colonial Ascidians Mytilus Sponges	Colonial Ascidians Sponges	Colonial Ascidians		
3	Dominants	Encrusting Bryozoans Eupomatus	Encrusting Bryozoans Eupomatus	Encrusting Bryozoans Eupomatus	Encrusting Bryozoans Mytilus	Mytilus	Mytilus
	Sub-dominants	Ciona Bugula Scrupocellaria	Ciona Styela Scrupocellaria	Styela Mytilus	Sponges	Sponges	Sponges
	Influents	Colonial Ascidians Mytilus Sponges	Colonial Ascidians Mytilus Sponges	Colonial Ascidians Sponges	Colonial Ascidians		
4	Dominants	Encrusting Bryozoans	Mytilus Ciona	Mytilus	Mytilus	Mytilus	Mytilus
	Sub-dominants	Ciona Mytilus		Ciona	Encrusting Bryozoans		
	Influents	Colonial Ascidians Erect Bryozoans	Colonial Ascidians Erect Bryozoans	Colonial Ascidians Erect Bryozoans			

TABLE I—*Continued*

Float number		September 21, 1944	October 23, 1944	November 22, 1944	December 21, 1944	February 13, 1945	March 26, 1945
5	Dominants	Ciona	Ciona	Ciona	Ciona	Ciona	Mytilus Encrusting Bryozoans
	Sub-dominants					Algae Hydroids	Ciona
	Influents					Mytilus Encrusting Bryozoans	Hydroids Colonial Ascidians
6	Dominants	Sponges	Sponges	Sponges Mytilus	Mytilus Sponges	Mytilus	Mytilus
	Sub-dominants	Styela Mytilus	Styela Mytilus	Styela		Sponges	Sponges
7	Dominants	Styela Mytilus	Mytilus	Mytilus	Mytilus Sponges	Mytilus	Mytilus
	Sub-dominants		Styela	Sponges			
	Influents	Bryozoans Sponges Pecten	Bryozoans Sponges Pecten	Bryozoans Pecten			
8	Dominants	Mytilus	Mytilus	Mytilus	Mytilus	Mytilus	Mytilus
	Influents	Styela Sponges	Styela Sponges	Styela Sponges	Styela Sponges	Styela Sponges	Styela Sponges

tember, had a population similar to that observed on float 2 in November, with relatively large numbers of *Mytilus* and *Ciona* on a bryozoan substratum. Within a month, *Mytilus* had largely displaced the bryozoans, and within three months, *Ciona* had also disappeared.

The *Ciona* community of float 5 remained virtually unchanged from September to February. By this time, however, the *Ciona* began to show signs of deterioration. They were heavily covered with algae and hydroids, and had many small *Mytilus* about their bases. In a few places, the ascidians had fallen from the float, to be replaced by encrusting bryozoans. In March, this change had progressed so far that *Mytilus* and the bryozoans could be regarded as the dominant organisms.

Floats 6 and 7 supported two types of sponge-*Styela* communities. These were rather rapidly displaced by *Mytilus*, however. Float 8 represented a well-developed *Mytilus* community and showed no change in composition during the six months of regular observation.

These observations suggest strongly that succession is operating here. The algal community is replaced by the bryozoans, and these in turn by *Mytilus*. *Ciona* and *Styela* communities are likewise replaced by *Mytilus*, but the *Mytilus* community is relatively stable. Further information bearing on this conclusion is available from the experimental studies to be reported in the next section.

Experimental Observations With Glass and Metal Surfaces

Experimental observations were made using glass plates, and supplementary information was available from a series of aluminum panels immersed for another purpose. The fact that the changes observed on the glass plates were entirely similar to those observed on wooden floats and metal plates suggests that the changes reported here are not dependent on the nature of the submerged surface. Coe (1932) and Coe and Allen (1937) concluded that the seasonal variations in abundance of populations or of different groups of organisms were the same on glass, concrete, and wood surfaces. They did find significant differences in the numbers and types of organisms on the different surfaces, however.

The glass panels used were four by nine inch rectangles of ordinary window glass in most cases; in a few experiments three by five inch panels were used. The metal plates were five by eight inch rectangles of aircraft aluminum (Alclad ST-37). The glass panels were at first exposed in a horizontal frame (Fig. 2) of redwood weighted with concrete. The frame was suspended from the laboratory pier, situated in the entrance channel to Newport Harbor about one-half mile from the outer end of the jetties protecting the harbor entrance. A rapid tidal flow passes this point twice daily, carrying with it abundant larvae from both the quiet-water fauna of the harbor and the open shore fauna of the jetties and adjacent rocks. In the second year of this study, with the glass plates, and throughout the work with the metal plates, a vertical suspension was used to facilitate handling of larger numbers of plates. The plates were suspended in slotted redwood crates, with a distance of one inch between plates. As the growth on the plates became heavier, this distance was increased to two inches. The plates were always suspended one or two feet below the level of the lowest tides.

All of the plates were examined regularly at intervals of two weeks, and then returned to the bay. A count was made, in most instances, of the numbers of each of the larger species on one surface (always the same for any plate). An estimate was also made of the area covered by each of the more abundant types of organism. Usually, this was done by a direct count of ten or more low-power microscopic fields distributed over the surface. When a plate was finally removed from the water, the organisms were carefully removed, sorted and weighed.

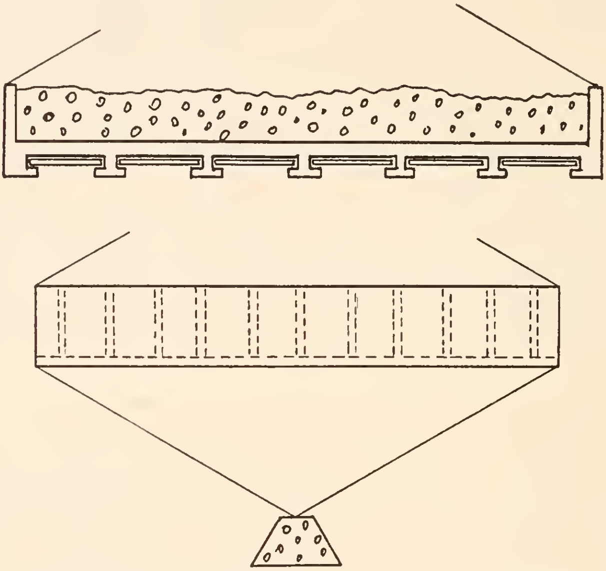


FIGURE 2. Horizontal and vertical suspension of panels.

The development of the algal community, and its transition to the bryozoan community could be followed very well on these plates. The first settlers were bacteria, diatoms, protozoans and, in the cooler months, hydroids. These were followed by the multicellular algae, especially *Ectocarpus*.

In the first months of this study it was observed that the larvae of bryozoans usually settled on the plates in quantity only after the second week of exposure, and sometimes did not settle until the fourth to sixth week. In order to verify this observation, careful counts were made during 1944 of the number of bryozoan colonies on each plate at two-week intervals. In this way, the minimum number of new settlers during any two-week period could be determined. Data obtained in this way are tabulated in Tables II, III, and IV for encrusting bryozoans, erect bryozoans and *Eupomatus*. The tabulation for the erect bryozoans omits the figures for the small semi-erect *Eucratea clavata;* representatives of this species settled in great numbers at irregular intervals, showing a behavior in this respect which was not at all comparable to the settlement of the other forms. The colonies were, moreover, rather short lived, dying often within a month of the original settlement.

During the first two weeks of exposure of any plate, the number of bryozoan and tubeworm settlements was usually less than during subsequent two-week periods. The preliminary period of light settlement was followed by a very heavy settlement in most cases. The growth of the early settlers, and in many cases, the large number of organisms settling during the maximal period, combined to reduce the available surface, and there was in consequence a very definite decrease in the number of organisms on the plate. By the time this decrease became evident, the plate was completely covered with bryozoans and tubeworms.

Table II

Number of new settlements of encrusting bryozoans on glass plates during successive two-week periods, 1944

Date examined	Date of original exposure														
	Dec. 20	Jan. 5	Jan. 17	Jan. 31	Feb. 14	Feb. 28	Mar. 12	Mar. 27	Apr. 27	May 9	June 8	July 6	Aug. 1	Sept. 11	Oct. 10
Jan. 5	0														
Jan. 17	0	0													
Jan. 31	0	0	0												
Feb. 14	3	0	0	0											
Feb. 28	10	1	0	0	2										
Mar. 12	28	5	2	3	—	0									
Mar. 27	30	26	30	14	5	0	0								
Apr. 8	0	7	—	18	8	10	2	2							
Apr. 26	24	62	60	120	64	60	57	9							
May 8					47	46	60	123	61						
May 24							12	13	17	14					
June 7									35	94					
June 21									11	53	30				
July 6											23				
July 17											48	25			
July 31											80	197			
Aug. 14												47	7		
Aug. 28												50	105		
Sept. 11												15	54		
Sept. 25													22	24	
Oct. 10														34	
Oct. 23														10	11

Figure 3 represents data derived from a metal plate first exposed March 28, 1944, and shows the changes in area covered by the algae, bacteria and hydroids on the one hand, and bryozoans on the other. The major increase in area occupied by the bryozoans occurred after the period of maximum settlement; the heaviest settlement occurred between the sixth and eighth weeks, while the rapid increase in area began between the tenth and twelfth weeks. This was in part the result of the manner of growth of bryozoan colonies. The number of new zooids formed increases directly with the number of zooids composing the colony, so that the rate of growth increases exponentially until crowding prevents further increase in the size of the colony.

TABLE III

Number of new settlements of erect bryozoans (exclusive of *Eucratea clavata*) on glass plates during successive two-week periods, 1944

Date examined	Date of original exposure												
	Jan. 17	Jan. 31	Feb. 14	Feb. 28	Mar. 12	Mar. 27	Apr. 27	May 9	June 8	July 6	Aug. 1	Sept. 11	Oct. 10
Mar. 27	0	0	0	0	0								
Apr. 8	4	0	0	0	0	0							
Apr. 26	11	10	10	0	5	0							
May 9	20	29	28	35	48	3	0						
May 24	5	4	6	—	17	18	0	0					
June 7	11	18	7	22	14	17	5	10					
June 21	21	27	36	24	—	27	72	44	2				
July 6								40	5				
July 17									28	12			
July 31									33	3			
Aug. 14									28	11	1		
Aug. 28										13	4		
Sept. 11											9		
Sept. 25											19	1	
Oct. 10											18	0	
Oct. 23													1

The length of time required for this sequence of events varied with the season of the year, but the character of the sequence did not vary. Thus, the plate exposed December 20 did not reach "saturation" with encrusting bryozoans until April, while the plate exposed May 9 had become "saturated" before the end of June (Table II). If we consider any particular two-week period, however, it is

TABLE IV

Number of new settlements of *Eupomatus* on glass plates during successive two-week periods, 1944

Date examined	Date of original exposure														
	Dec. 20	Jan. 5	Jan. 17	Jan. 31	Feb. 14	Feb. 28	Mar. 12	Mar. 27	Apr. 27	May 9	June 8	July 6	Aug. 1	Sept. 11	Oct. 10
Jan. 5	0														
Jan. 17	0	0													
Jan. 31	0	0	0												
Feb. 14	2	0	0	0											
Feb. 28	15	4	0	0	0										
Mar. 12	17	10	1	0	0	0									
Mar. 27	7	1	6	2	0	0	0								
Apr. 8	8	14	13	9	1	2	0	0							
Apr. 26	1	1	10	2	4	2	0	0							
May 8	1	3	2	—	0	1	1	0	0						
May 24					2	—	—	0	0	0					
June 7							3	1	2	0					
June 21							0	3	0	7	8				
July 6							7	4	12	20	11				
July 17											4	0			
July 31											2	5			
Aug. 14											—	5	0		
Aug. 28											14	39	15		
Sept. 11													11		
Sept. 25													—	11	
Oct. 10													4	3	
Oct. 23														3	0

evident from Tables II to IV, that in general, the most recently exposed plates received lighter settlements of the three types of organisms concerned than did those which had been in the water somewhat longer. Evidently changes occurred following immersion which rendered the plate more suitable for settlement of bryozoans and tubeworms than was the clean surface. These changes occurred more rapidly in the warmer months.

Two experiments were performed to test this hypothesis, and to throw more light on the nature of the changes involved. ZoBell and Allen (1935) and Coe and

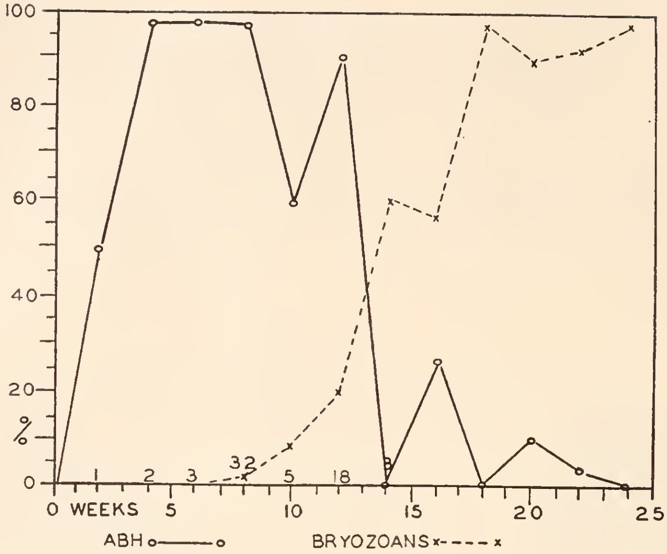


FIGURE 3. Relative areas, in per cent, covered by algae, bacteria, and hydroids (A B H), and bryozoans on an aluminum panel exposed March 28, 1944. The figures along the abscissa represent number of new settlements of bryozoans in each two-week period.

Allen (1937) have suggested that bacterial film is an important feature in the establishment of sedentary forms on a submerged surface. In the first experiment (Table V), ten three by five inch glass plates were sterilized. Two were then exposed in the bay, two were left in sterile sea water, two in a sterile solution of 0.1 per cent peptone in sea water, and two were placed in a solution of 0.1 per cent peptone in water freshly drawn from the bay. After four days, by which time a vigorous bacterial population had developed in the bay water solution, all ten

TABLE V

Settlement of organisms on pretreated glass plates, June 6–10, 1944. Duration of treatment, 4 days. Figures represent number of organisms or colonies

Treatment:	First series (3 days immersion)			Second series (4 days immersion)		
	Hydroids	Bryozoans	Ascidians	Hydroids	Bryozoans	Ascidians
Sterile sea water	43	0	0	68	4	0
Sterile sea water + 0.1% peptone	53	2	2	44	0	0
Bay water + peptone (bacterial)	150	2	2	174	4	0
Immersal in bay 4 days	53	11	11	83	14	0
Sterile plate	47	2	2	52	3	0

plates were placed in the bay. The results are presented in Table V. The hydroids evidently settled more abundantly on the bacteria-coated plates then on the others, but the bryozoans and ascidians were not influenced by the bacterial coating. Rather, they settled more abundantly on the plates which had been in the bay longest; these plates had a more abundant diatom population than did the others.

A second similar experiment was carried out in the fall, with daily observations during several weeks of exposure, and careful determinations of the bacterial and algal populations. Diatoms appeared on the plates in small numbers within the first two to four days in the bay (Table VI). For a period of two to three weeks, however, the diatoms covered less than 5 per cent of the surface. This period was

TABLE VI

Settlement of organisms on treated panels, October 21 to November 24, 1944. The figures represent the per cent of the area of one side of the panel covered by bacteria, diatoms, and protozoans respectively, and total number of organisms or colonies on one side of the panel for the larger organisms (bryozoans, annelids, ascidians).

Duration of treatment:		5 days				18 days			
Treatment:		Bay	Sterile	Sterile sea water + peptone	Fresh bay water + peptone	Bay	Sterile	Sterile sea water + peptone	Fresh bay water + peptone
Days after treatment	Organism								
7	Bacteria	0.3%	0.2%	0.3%	1%		1%	2%	11%
	Diatoms	4%	1%	1%	1%	80%	0.3%	1%	1%
	Protozoans	+	+	+	+		0.3%	1%	+
	Bryozoans:								
	Membranipora	6	3	1	9	0	1	0	0
	Other encrusting forms	0	0	2	0	8	1	2	3
	Annelids	0	1	0	3	3	0	0	8
	Ascidians	1	0	5	3	0	0	3	7
16	Bacteria	1%	1%	2%	1%		5%	7%	8%
	Diatoms	35%	11%	8%	16%	51%	5%	4%	18%
	Protozoans	6%	1%	0.1%	6%		3%	5%	6%
	Encrusting bryozoans exc. Membranipora	13	6	11	14	14	8	9	17
	Annelids	1	2	2	3	8	0	1	9
	Ascidians	2	2	9	5	0	2	1	0
Time of maximum increase, days after immersion in bay	Diatoms	19	14	20	14	19	16	14	16
	Encrusting bryozoans exc. Membranipora	19	14	22	16	21	16	16	16

followed by a relatively sudden increase in the number of diatoms, until 25 per cent to 80 per cent of the plate was covered. The reason for this delay is not clear. During the first two to three weeks of exposure, bacteria and protozoans as well as diatoms settled on the plates. That bacteria were not concerned in the eventual diatom outburst is indicated by the fact that the bacteria-coated plates (bay water and peptone) showed no difference from the other plate in the time of the outburst. About 5 per cent of the area of the plate which had been immersed in bay water plus peptone for five days was covered with bacteria when the plate was immersed in the bay; the eighteen day plate was covered to an extent of about 11 per cent. This coating was largely lost within a few days, however. It is noteworthy that, although the time of maximum increase of the diatoms was not affected by the presence of bacteria, larger populations of diatoms eventually developed on the bacteria-coated plates than on the other plates. Algae other than diatoms were not important in this experiment; *Ectocarpus, Enteromorpha, Cladophora, Scytosiphon, Pterosiphonia* and *Lophosiphonia* were noted, but did not appear in quantity until some time after the diatom increase.

The data available from this study are not sufficient to establish a succession within the algal community. Wilson (1925), however, has reported a definite sequence, involving diatoms, hydroids and filamentous algae, on rocks at La Jolla. It is quite possible that careful study over a longer period would reveal a similar situation here.

It appears that the relatively heavy growth of diatoms on the bay water plus peptone plates is correlated with a correspondingly heavy settlement of bryozoans. Whether there is a direct causal relation between diatom growth and bryozoan settlement is uncertain. However, the maximum period of bryozoan settlement never preceded, and usually followed, the period of maximum diatom increase in the experiment of Table IV.

The encrusting bryozoan *Membranipora tuberculata,* which normally inhabits the stipes of kelp, occurred on the experimental plates only on one occasion, and remained only a short time. Most of the colonies fell from the plate within the space of a month. Unlike the other bryozoans, however, this species showed definite preference for the bacteria-coated plate.

These experiments suggest, then, that the important change which occurs on plates favoring bryozoan settlement, is the growth of diatoms. In view of this conclusion and of the fact that the bryozoan community, throughout the course of this study, has been observed to form only on surfaces previously supporting an algal growth, we may say that a definite succession is established.

The observations on the glass plates provide little evidence concerning the other communities, but two instances are worthy of mention. The development of a *Styela* community from a bryozoan community was noted in one instance, on a plate exposed horizontally in March 1943. The algal coat which developed upon exposure was displaced by bryozoans before the end of June. In September, however, specimens of *Styela* which had settled in July had become so large as to dominate the community. The remaining bryozoans gradually lost ground and fell from the plate, leaving *Styela* as the principal organism present. The fact that *Styela* was always found growing out of a substratum of old bryozoan colonies on the floats examined in the course of this study indicates that this sequence probably occurs frequently.

The second instance concerns the formation of a *Balanus* community. The plate concerned was exposed horizontally in August 1943. The algal community formed very rapidly, and in addition, within two weeks, larvae of *Eupomatus*, encrusting bryozoans, *Pecten* and *Balanus* had settled in large numbers. In the ensuing competition for space, the barnacles emerged victorious. In September, there were more than two hundred barnacles on the exposed side of the plate, covering the surface almost completely. An equal number of *Eupomatus* occupied the spaces between the barnacles, but encrusting bryozoans were not abundant. During subsequent months, however, growth of the bryozoans was continuous, and by December, the barnacles were almost completely covered by the rapidly growing bryozoans.

Discussion and Conclusions

In order to make a satisfactory analysis of the phenomena described in the preceding sections, it would be necessary to know (a) the breeding seasons of the organisms involved, (b) the normal duration of life of each of the important organisms, (c) the length of the free-swimming larval period in each case, and (d) the nature of the surfaces to which such free-swimming larvae will attach. We do not have such information in most cases. Nevertheless, it is possible to make some interpretations on the basis of the available information.

It was stated at the outset that the basic problem is that of distinguishing between seasonal progression and true succession. Several examples of progression were noted at Beaufort, N. C. by McDougall (1943). The organisms which settled during the winter were, for the most part, dead or moribund by spring, and were consequently replaced by organisms breeding chiefly in the spring. There is some reason to expect that seasonal progression may be less important in Newport Harbor than at Beaufort. The annual range of monthly mean temperatures at Beaufort is 23° C., from 5.5° in February to 28° in July. The annual range in Newport Harbor is only 5° C., from a low of 14.1° in February to a high of 19.2° in July. The breeding seasons of most of the organisms involved in the sequences described here extend through most of the year. Certainly algae, bryozoans and mussels have been observed to settle during every month of the two years covered by this study.

In the present study, it seems probable that the algal community, and most probably the diatoms comprising the basis of that community, provide favorable conditions for the settlement of bryozoans. Bryozoans settled in quantity only after the development of a fairly vigorous algal community. Moreover, in the experimental test described in Table VI, bryozoan settlement was definitely correlated with the settlement and growth of diatoms. There remains the possibility that some common factor favored settlement of both diatoms and bryozoans, the former remaining "dominant" only until the slower but persistant growth of the latter displaced them. It is difficult to rule out such common factors, but it appears unlikely that chemical alterations in the glass on exposure to sea water are involved. The plates used in this particular experiment had previously been immersed in the bay for two months. They were then scrubbed with a brush in tap water, wrapped in paper and sterilized in an autoclave. The experimental plates were soaked in three liters of solution for several days as noted in the table,

TABLE VII

Weights of organisms removed from glass and aluminum panels, 1943–44
(+ sign indicates organisms present in amounts of less than 1 gram)

Surface:		Glass													Aluminum		G	G	Al
Duration of exposure, weeks:		4			8				12	20					26	28	32	36	
Date of initial exposure:		Mar. 12 '43	Apr. 4 '43	Dec. 20 '43	Mar. 12 '43	May 7 '43	July 5 '43	Dec. 20 '43	Dec. 20 '43	Dec. 20 '43	Jan. 31 '44	Feb. 28 '44	Mar. 27 '44	Apr. 27 '44	Sept. 21 '43	Mar. 28 '44	Jan. 17 '44	Dec. 20 '43	Dec. 14 '43
Total weight of organisms, grams:		20	56	9	23	67	18	12	19	117	204	302	174	157	83	194	193	180	128
Per cent of weight contributed by:																			
Algae, Bacteria, Hydroids, Debris:		100	85	100	87	76	28	100	81	2	1	30	9	29	59	21	18	15	11
Bryozoans	Encrusting forms				+	1	29	+	5	57	54	34	35	41	30	63	27	34	66
	Erect forms			+		9	3	+	+	21	22	11	25	16	2	6	28	17	1
Annelids	Serpulids					+	14	+	+	10	2	1	+	3	6	1	5	8	1
	Others		8		+	1		+	3	1	5	1	1	1	1	1	1	1	2
Crustaceans	Erichthonius		4	+			3	+	11	8	5	21	14	6	1	3	10	19	5
	Balanus						14			+		+	+	+	1	+			1
	Others		3	+	+	1	3	+	+		+	+	+	+	+				+
Mollusks	Pecten				+	10	6	+				+	1		+	+	+	+	+
	Mytilus															3			
	Saxicava				+	+				+	+	+	4	2	+	1	8	4	8
Sponges											5	+			1	+	+	+	+
Ascidians				+	+		+			1	5	1	11	1		1	2	2	5

while the control plates were placed directly in the bay. The same sequence of organisms is apparent on both experimental and control plates, and the periods of time involved are not significantly different.

The data in Table VII are of interest in this connection. It is apparent that the organisms listed fall into four classes: (1) Those which appear in abundance on all plates, but most abundantly on those exposed for the shortest periods (algae, etc.). (2) Those which appear only on plates exposed more than four weeks, and most abundantly on plates exposed twenty weeks or longer (bryozoans, serpulid worms). (3) Those which appear in measurable quantities only on plates exposed twenty weeks or longer, and are not abundant even on plates exposed as long as thirty-six weeks (*Mytilus, Saxicava,* sponges, ascidians). (4) Those which appear irregularly, without relation to the duration of exposure (annelids, *Balanus, Erichthonius* and other crustaceans, *Pecten*).

It is particularly significant that the dominant organisms of the primary communities involved in the sequence described earlier—viz. algae, bryozoans, ascidians and mussels—fall into separate categories on this basis, and that the sequence here is the same as that observed in the sequence of communities. It appears that the settlement of ascidians certainly and mussels probably is favored by the existence of a thriving bryozoan community.

In any event, there is no evidence that seasonal progression is involved to a significant extent in the algae-bryozoan-*Mytilus* sequence. A plate exposed in December went through the same sequence as did one exposed in March or April; the time relations varied, but the sequence did not. And in the absence of a seasonal progression, it is difficult to avoid the conclusion that true succession is involved.

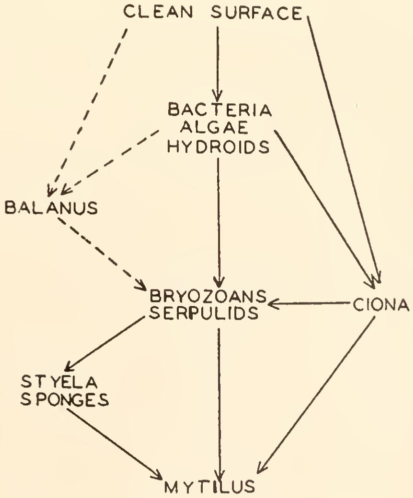


FIGURE 4. Sequence of dominant organisms on surfaces exposed in Newport Harbor.

In many studies of the life histories of sedentary organisms, estimates of the season of settling have been based on the number of new settlers on a plate exposed for a brief period. It is evident from the results reported here that such estimates may be unreliable if succession is involved in the settlements under consideration. Thus, plates exposed for four weeks or less in Newport Harbor in the winter months would receive few or no settlements of bryozoans, despite the fact that settling larvae are present in the water during these months. It is important, therefore, that studies of this sort take into consideration the question whether succession is occurring.

With the evidence presented in this paper, we can make a number of suggestions as to the possible factors involved in the events described. The sequence is depicted in Figure 4. A newly exposed surface is first colonized by bacteria, algae and, in some seasons, hydroids. The development of these forms provides a favorable surface for establishment of bryozoan colonies, and also for the settlement of serpulid larvae. The vigorous growth of the bryozoans eventually displaces the algae and hydroids. The resulting bryozoan community in turn provides a favorable basis for the attachment of *Mytilus* larvae. The growth of the mussels effectively covers the whole surface of the bryozoan community, the members of which eventually perish from lack of food, oxygen, etc. Seasonal factors, involving the settlement of ascidian or barnacle larvae in tremendous numbers, may introduce variations into this sequence. *Ciona* may sometimes colonize a clean surface, or one covered with algae, to such an extent that the bryozoans are unable to maintain their foothold. *Styela* apparently settles only on bryozoan substrata, but may become established before *Mytilus,* and hence a community dominated by *Styela* may follow the bryozoan stage. Sponges are frequently associated with *Styela.* Both *Ciona* and *Styela* communities are eventually displaced by *Mytilus* which at present represents the climax in the float-bottom associations of Newport Harbor.

SUMMARY

1. The sedentary communities characteristic of float bottoms in Newport Harbor, California, are described.

2. The most important communities at present are dominated, respectively, by algae, bryozoans, *Ciona intestinalis, Styela* sp. and *Mytilus* sp.

3. These communities represent stages in an ecological succession.

4. The algal community appears first on freshly exposed surfaces, to be followed usually by a bryozoan community.

5. The bryozoans prominent in the bryozoan community settle more readily on surfaces supporting a vigorous growth of diatoms and other algae than on clean surfaces.

6. The community dominated by *Mytilus* constitutes the climax at present.

7. *Mytilus* has been observed to settle only on surfaces bearing a bryozoan, *Ciona* or *Styela* community.

8. The establishment of *Ciona* or *Styela* communities appears to depend in part on seasonal factors.

ACKNOWLEDGMENT

I should like to express my appreciation of the helpful criticisms and suggestions of Professors W. R. Coe, M. W. Johnson, G. E. MacGinitie and A. C. Redfield. Miss Margaret L. Campbell rendered invaluable technical assistance during a part

of this work. I am indebted to Dr. Raymond C. Osburn for identification of the bryozoans.

LITERATURE CITED

Coe, W. R., 1932. Season of attachment and rate of growth of sedentary marine organisms at the pier of the Scripps Institution of Oceanography, La Jolla, California. *Bull. Scripps Inst. Oceanogr. Tech. Ser.*, **3**: 37–86.

Coe, W. R., and W. E. Allen, 1937. Growth of sedentary marine organisms on experimental blocks and plates for nine successive years at the pier of the Scripps Institution of Oceanography. *Bull. Scripps Inst. Oceanogr. Tech. Ser.*, **4**: 101–136.

Hewatt, W. G., 1935. Ecological succession in the Mytilus californianus habitat as observed in Monterey Bay, California. *Ecology*, **16**: 244–251.

Kitching, J. A., 1937. Studies in sublittoral ecology II. Recolonization of the upper margin of the sublittoral region. *J. Ecology*, **25**: 482–491.

McDougall, K. D., 1943. Sessile marine invertebrates at Beaufort, North Carolina. *Ecol. Monogr.*, **13**: 321–374.

Moore, H. B., 1939. The colonization of a new rocky shore at Plymouth. *J. An. Ecology*, **8**: 29–38.

Moore, H. B., and N. G. Sproston, 1940. Further observations on the colonization of a new rocky shore at Plymouth. *J. An. Ecology*, **9**: 319–327.

Shelford, V. E., 1930. Geographic extent and succession in Pacific North American intertidal (Balanus) communities. *Publ. Puget Sound Biol. Sta.*, **7**: 217–224.

Wilson, O. T., 1925. Some experimental observations of marine algal successions. *Ecology*, **6**: 303.

ZoBell, C. E., and E. C. Allen, 1935. The significance of marine bacteria in the fouling of submerged surfaces. *J. Bacteriol.*, **29**: 239–251.

A COMPARISON OF THE EFFECTS OF CYANIDE AND AZIDE ON THE DEVELOPMENT OF FROGS' EGGS [1]

S. SPIEGELMAN [2] AND FLORENCE MOOG

Department of Zoology, Washington University, Saint Louis 5, Missouri

Loeb's (1895) observations that the eggs of *Fundulus heteroclitus* are capable of considerable development under anaerobic conditions has since been extended to various amphibian embryos. Brachet (1934), in confirming the possibility of anaerobic development for *Rana temporaria* eggs, reported also that cyanide is similar to anaerobiosis in its effects on embryogenesis. Eggs placed in cyanide immediately after fertilization were arrested in the late blastula, but those placed in cyanide after gastrulation had begun would continue to the formation of a complete blastopore. Later stages were increasingly sensitive to cyanide. Although it has generally been thought that the arrests of development caused by cyanide are due to inhibition of the cytochrome oxidase of the Warburg-Keilin system (Keilin, 1933), it might be inferred from the recent work of Holtfreter (1943) that the repressive effects of cyanide solutions result merely from their alkalinity. It will be shown in this paper however that only post-mortem effects are influenced by the pH of the cyanide solution, the actual stoppage resulting from the presence of the toxic radical itself.

In 1936 Keilin reported in detail on another specific inhibitor of cytochrome oxidase, sodium azide (NaN_3). On the basis of these experiments NaN_3 and NaCN have in some cases been used interchangeably. Philips (1940), in comparing the developmental sensitivities to anaerobiosis of pelagic and non-pelagic fish eggs, employed both NaCN and NaN_3. He found that Fundulus eggs before the end of gastrulation are capable of extensive development in concentrations of both cyanide and azide which completely and almost immediately inhibit pelagic eggs. Except for the higher concentrations required in the case of NaN_3 he could demonstrate no difference between the effects of the two reagents. Recently Barnes (1944) tested the same reagents on the development of *Rana pipiens*. The results with cyanide confirmed the earlier observations of Brachet (1934). While no detailed data are given, the effects of azide were apparently found to completely parallel those of cyanide, for Barnes states: "Eggs exposed to M/100 NaN_3 at pH 7.0 are able to develop to the gastrula stage. Gastrulation never occurs in the presence of azide." Lower concentrations (M/1000) did not stop gastrulation although the eggs developed at a slower rate.

The present authors (Moog and Spiegelman, 1942), while investigating the relation between regeneration and metabolic activity, demonstrated a specific difference between the effects of azide and cyanide on hydranth reconstitution in Tubularia. Azide could inhibit regeneration at concentrations which did not sensibly

---

[1] Aided by a grant from the Rockefeller Foundation.

[2] Present address: Department of Bacteriology and Immunology, Washington University Medical School, Saint Louis, Missouri.

affect respiration whereas cyanide caused parallel depressions in rates of regeneration and of respiration. Subsequent analysis (Spiegelman and Moog, 1944) of the differential effects of these two agents on the mass and time of appearance of the new hydranth emphasized the difference in their activities.

In the fall of 1941 the authors undertook a comparison of the effects of NaCN and NaN_3 on the development of *Rana pipiens*.[3] The results obtained disagree in certain respects with those reported by Barnes (1944). Azide was found to be completely effective in stopping morphogenesis at all stages of development, including those between fertilization and gastrulation which are not inhibited by cyanide. In an effort to discover the cause for the disagreement these experiments were repeated recently under conditions closer to those employed by Barnes. Our earlier results were confirmed and the discrepancy remains unresolved. No direct comparison with the findings of Philips (1943) is possible, not only because of the difference in material but also because the highest concentration he employed was below the one we found to give consistent inhibitions.

The results will be detailed and the difference obtained between the effects of azide and cyanide will be discussed in the light of recent findings on azide inhibitions of anaerobic synthetic processes.

General Methods and Materials

Eggs of *Rana pipiens,* obtained by injection of pituitary glands, were expressed and artificially fertilized. After swelling of the jelly the eggs were cut up into small groups in 10 per cent Ringers solution adjusted to the desired pH with phosphate buffer. Stages were determined according to the schedule of Pollister and Moore (1937) and are so numbered in the present paper. The eggs were stripped from the jelly with fine forceps before being immersed in the experimental solutions.

All hydrogen ion concentrations were determined with a glass electrode after the reagents were added. Where temperature control is indicated the designated temperature was held within ± 0.2° C. Other experimental details will be found in the appropriate places of the text.

Experimental Results

The effects of azide and cyanide on development

Keilin (1936) as well as subsequent investigators demonstrated the critical influence of pH on the effectiveness of azide as a respiratory inhibitor. Using the isolated Warburg-Keilin system as well as yeast cells Keilin obtained maximal effects at about pH 6.3 when the azide was used in concentrations of 0.001 and 0.002 molar. In the experiments to be described in the present section azide solutions were adjusted to pH 6.6. The concentration chosen for study was 0.005 molar, since parallel experiments on the effects on respiration (see Spiegelman and Steinbach, 1945) indicated maximal effects at this concentration on respiratory rate. The same can be said for development, for 0.005 M azide yields completely effective inhibition. All controls for the azide experiments were similarly adjusted to pH 6.6. In the case of cyanide both experimentals and controls were run at pH 8.4. The controls at pH 8.4 did not differ detectably in rate of development from those at pH 6.6. Every experimental set had its own control and both were thus handled exactly the same number of times and in the same fashion. This avoided the relatively more frequent handling and examination of the controls which would have been necessary if one set of eggs were the controls for a larger number of experimentals. For convenience in observations all of the present experiments were done at 15.2° C. in a cold room. To avoid the accelerating and decelerating effects of changing temperatures during development (see Ryan, 1943) the eggs were kept at 15.2° C. in 10 per cent Ringers until they reached the stage it was desired to test. They were then transferred to the approximate solutions previously brought to the same temperature. The cyanide solutions were freshly prepared and renewed every 12 hours during the course of an experiment; the experimental solutions were kept in stacked fingerbowls, with an empty bowl covering the top member of the stack.

---

[3] These studies were carried out in the laboratories of the Department of Zoology, Columbia University, New York.

For the purposes of comparison with the azide experiments, the results with cyanide in the early stages are reproduced in Table I. They do not differ in essentials from those reported by Brachet (1934). Eggs placed in cyanide early in development, although delayed as compared with controls, continue to develop up to gastrulation. The later the stage at which they are subjected to cyanide the closer is the approach to gastrulation; they do not however actually begin to gastrulate. Eggs in early stage 9 will continue to segment until the cells at the vegetal pole are quite minute but will evidence no signs of dorsal blastopore lip formation. If however the invagination has already started cyanide will not immediately stop it and the eggs may proceed to the formation of a complete blastopore before ceasing activity. Later stages become increasingly sensitive to cyanide.

TABLE I

The effects of cyanide on development at pH 8.4 at 15.2° C. These experiments were done in 1941–2 on material obtained from Vermont. The numbers represent the developmental stages as described under Methods.

Stage at start of experiment	Solution	Hours after immersion											No. of eggs
		4	8	12	18	24	35	45	55	75	95	120	
Uncleaved	0.001 M	2	3		6	8	8	8	8				210
Uncleaved	Control	3	3		8	8	10	11	12				210
3	0.001		4		6	7	8	8	9	9	9		200
3	Control		6		8	9	10	11	12		16		200
6	0.001		7			8	8	8	8	9	9	9	160
6	Control		8			10	11	12	12	13	16	18	160
9	0.001		9		9		9	9					200
9	Control		10		11		12	13		16			200
10	0.001			10		10		11		12		12	200
10	Control			11		12		14		16		18	200

The results obtained with azide are summarized in Table II. It is immediately evident that all stages are azide sensitive, including the early ones which are not effectively inhibited by cyanide. It might be noted that under these experimental conditions the cessation of developmental activity on immersion in azide solution is, as far as can be determined, abrupt and immediate. This was easily ascertained in the early cleavage stages since no further cleavage was observed. Although the observations are more difficult in the later stages, careful examination failed to reveal any development subsequent to treatment with azide. If the eggs are removed within 30 minutes after being placed in the azide solution and thoroughly washed they can proceed with their development.

TABLE II

The effect of azide on development at pH 6.6 at 15.2° C.; 1941–2, material from Vermont. The numbers represent the developmental stages as described under Methods

Stage at beginning	Solution	Hours after immersion											No. of eggs
		4	8	12	18	24	35	45	55	75	95	120	
Uncleaved	0.005 M	1		1			1	1	1	1	1	1	100
Uncleaved	Control	3		5			10	11	12	14	16	18	100
6	0.005 M		6			6	6	6	6	6	6	6	150
6	Control		7			9	10	11	12	14	16	18	140
7	0.005 M		7	7		7		7	7	7	7	7	90
7	Control		9	10		11		12	14	16	17	18	90
9	0.005 M	9			9			9		9	9	9	110
9	Control	10			11			13		16	17	18	110
10	0.005 M			10			10			10	10		160
10	Control			11			13			17	18		160
11	0.005 M		11				11		11		11		85
11	Control		12				14		16		18		85
12	0.005 M					12			12				105
12	Control					14			16				105
13	0.005 M		13			13	13		13				120
13	Control		14			16	17		18				120
14	0.005 M				14		14	14			14		90
14	Control				16		17	18			19		90
16	0.005 M			16			16		16	16			110
16	Control			17			18		18	19			110
17	0.005				17	17	17	17	17				60
17	Control				18	18	18	18	19				60

Barnes' (1944) experiments with azide were done at higher temperatures, concentrations, and pH than those described above. Accordingly when the azide experiments were repeated they were done at room temperature (ca. 25° C.), at pH 7.4 and 8.3 (i.e. with and without added hydrochloric acid), and with concentrations up to 0.01 M. The results of these experiments are given in Table III. At both hydrogen ion concentrations, 0.01 M azide caused immediate arrest in all pregastrular stages. The 0.005 M concentration used in the early experiments was retested under these conditions and found to give exactly the same results as previously obtained. Controls kept in Ringers buffered at the experimental pH developed normally in all cases, and are not reported in the table.

TABLE III

The effect of azide on development at 25° C.; 1944–5, material from Wisconsin

Stage at immersion	Conc. (Molar)	pH	Stage at arrest
1	0.001	7.4	9*
1	0.001	8.3	9*
1	0.005	7.4	1
1	0.005	8.3	1
1	0.01	7.4	1
1	0.01	7.4	1
1	0.01	8.3	1
7	0.005	7.4	7
7	0.01	7.4	7
9	0.005	7.4	9
9	0.005	7.4	9
11+	0.01	8.3	12−
13	0.01	8.3	13

* There was no delay in reaching this stage.

It is clear that we can offer no support to Barnes' statement that at the concentration and pH she employed, azide, like cyanide, permits eggs to develop to gastrulation.

The effect of NaCN on development at different pH values

Holtfreter (1943) presented evidence showing that the disruptive effects of strong cyanide solutions (0.1 M to 0.0015 M) can be imitated by potassium hydroxide solutions of equal pH. Although the author did not specifically claim that the oxidation-repressing effects of cyanide are to be regarded as completely irrelevant to its influence on development, it nevertheless seemed advisable to us to clarify the points which were left in an indecisive state by Holtfreter's work. This we did, in our 1944–1945 series of experiments, both by comparing the effects of NaCN solutions brought to pH 7.2 with HCl with those at pH 9.6–9.8, and by determining the effects of solutions of either NaOH or KOH at pH 9.8. The tests were made at about 25°; the cyanide solutions were changed three times daily, the hydroxide solutions once daily.

The results of the NaCN tests completely confirmed our earlier findings (Table IV). The stage in which development was stopped, and the speed with which that stage was reached, was in all cases the same in solutions of equal concentration at both low and high pH. Only after the egg had been in an arrested stage for more than 12 hours did a difference between the two pH's become evident. At high alkalinity the pigment became streaked, the surface disintegrated, and the egg was in the majority of cases reduced to a loose, fuzzy mass of cells within 36 hours; at low alkalinity the surface was only moderately eroded after 72 hours.

Table IV

The effects of NaCN at pH 7.2 and 9.8; 1944–5, material from Wisconsin

Stage at immersion	Conc. (Molar)	pH 7.2		pH 9.6–9.8	
		Stage at arrest	Later effects	Stage at arrest	Later effects
1	0.003	Not tested		7	Egg swollen and surface severely depigmented after 36 hrs.
1	0.006	Not tested		7	
7	0.003	9	Marked depigmentation after 40 hrs.	9	Depigmentation after 20 hrs., surface disintegrated after 36 hrs.
7	0.006	9		9	
9	0.003	11	Blastopore lip disappeared within 20 hrs. after forming	11	Blastopore lip also disappeared. Surface completely disintegrated after 24 hrs.
9	0.006	11		11	
1	0.004	7	Surface became mottled but did not disintegrate within 72 hrs.	7	Complete disintegration within 24 hrs.
1	0.004	7	Egg swelled to twice its normal diameter but did not disintegrate within 96 hrs.	7	Complete disintegration within 24 hrs.
8	0.004	9	Surface became mottled and egg swelled somewhat, but did not disintegrate within 88 hrs.	9	Surface became mottled within 24 hrs., complete disintegration within 38 hrs.

The studies with hydroxides revealed that *Rana pipiens* eggs can develop from fertilization to the stage of tail-fin circulation (stage 22, at which they were discarded) at pH 9.8 (i.e., 2.5×10^{-4} M). Stage 22 was also achieved uneventfully if the eggs were immersed in the hydroxide solutions at the stage of the morula (S7), late blastula (S9), mid-gastrula (S11), neurula (S14), muscular movement (S18); in the last two cases the vitelline membrane was removed before the embryos were placed in the alkali solutions. In complete contradiction to Holtfreter's finding that eggs disintegrate in the morula stage in KOH solutions of pH 9.0 to 9.4, we did not observe either retardation or abnormality of development. In three experiments with NaOH and two with KOH, we obtained identical results. Thus we may conclude that the suppressive action of NaCN (or KCN) on living egg is due to the poisonous effect of the CN component.

Discussion

Both azide and cyanide are effective inhibitors of respiration in the early as well as in the later stages of development (Barnes, 1944; Spiegelman and Steinbach, 1945). The fact that cyanide cannot inhibit at any stage before gastrulation whereas azide can inhibit at all stages, cannot be explained on a respiratory basis. This is even more pointedly demonstrated by the capacity of eggs to develop to gastrulation under anaerobic conditions. The ability of cyanide to depress respiratory rates at all stages clearly proves that it gets into the cells of the early embryos, and consequently a difference in permeability cannot be invoked to explain the difference between the effects of azide and cyanide on development. It is clear from these experiments that, at least in the early stages, NaN_3 is inhibiting some cyanide-insensitive process necessary for development.

Recent work has served to question conclusions drawn from Keilin's earlier experiments that azide and cyanide are essentially equivalent inhibitors of the Warburg-Keilin system. Stannard (1939) showed that cyanide inhibited the respiration of both resting and active muscle while azide affected active muscle only. Armstrong and Fisher (1940) demonstrated that azide and cyanide behave differently in inhibiting the enzymes controlling the frequency of the embryonic fish heart-beat. Differences in cyanide and azide inhibitions of tissue respiration led Korr (1941) to postulate the existence of different pathways of respiration in resting and stimulated tissues. Ball (1942) suggested different oxidation-reduction potentials for the Atmungsferment-cyanide and Atmungsferment-azide compounds as an explanation of the different effects of the two inhibitors. Winzler (1943), after subjecting the kinetics of the respiratory inhibition by cyanide and azide in yeast to a careful examination, came to the conclusion that cyanide inhibited yeast respiration by three different pathways: (1) by combining with oxidized Atmungsferment; (2) by increasing the apparent KO_2 of reduced Atmungsferment; and finally (3) by combining with the enzyme which controls the rate-limiting step of the rate of respiration. Azide on the other hand exhibited only one type of inhibition, namely, combination with oxidized Atmungsferment.

Aside from these studies on respiration, others have been made on assimilatory activity of microorganisms. Barker (1936) and Giesberger (1936) showed that suspensions of bacteria could under certain circumstances synthesize carbohydrate from various substrates. Clifton (1937) studied the effect of azide on these syntheses and found them to be completely inhibited. In the presence of azide external substrate was completely oxidized. Clifton and Logan (1939) extended these findings and showed that it was possible to differentially inhibit assimilatory processes with both NaN_3 and 2, 4-dinitrophenol. Winzler (1940), working with acetate assimilation in yeast, showed that low concentrations of azide, cyanide, or 2, 4-dinitrophenol prevented assimilation. Azide was also shown by Winzler (1944) to prevent the anaerobic assimilation of glucose by yeast without interfering with its fermentation. Winzler, Burk, and du Vigneaud (1944) found that azide in concentrations of 10^{-4} and 10^{-3} molar inhibits completely the anaerobic assimilation of ammonia.

These experiments show that azide, and in certain instances cyanide, can inhibit synthetic processes which are essentially anaerobic in nature and not connected with the Warburg-Keilin system. It seems most probable that it is this sort of inhibitory activity which is involved in the ability of azide to stop embryonic development. Unfortunately, with the exception of Winzler's (1940) study of acetate assimilation no detailed comparison between the effects of azide and cyanide on synthetic processes has been published. In view of the results reported in the present paper one would venture to predict that such differences will be discovered. It may be noted that one such difference has been found in the case of adaptive enzyme formation in yeast, which is azide sensitive but is not inhibited by cyanide (Spiegelman, 1945). A suggestive finding has been reported recently by Meyerhof (1945), who prepared a solution of adenylpyrophosphatase from yeast by supersonic vibration and found it insensitive to cyanide but highly sensitive to azide. This enzyme, involved as it is in transphosphorylation, might conceivably be a part of the azide sensitive anaerobic synthetic processes.

SUMMARY

Previous observations that amphibian eggs can develop up to the beginning of gastrulation in cyanide solutions have been confirmed on eggs of *Rana pipiens*. The effect of cyanide is independent of pH, and eggs can develop into tadpoles in 2.5×10^{-4} molar NaOH or KOH solutions at pH 9.8.

Azide has been found to arrest development immediately in all stages from fertilization to tail-bud formation. The effect is the same from pH 6.6 to pH 8.3.

These differences are discussed in the light of recent studies on the effects of azide and cyanide on respiratory, assimilatory, and phosphorylative processes.

LITERATURE CITED

Armstrong, C. W. J., and K. C. Fisher, 1940. A comparison of the effects of respiratory inhibitors azide and cyanide on the frequency of the embryonic fish heart. *J. Cell. Comp. Physiol.*, **16**: 103–112.

Ball, E. C., 1942. Oxidative mechanisms in animal tissues. *A Symposium on Respiratory Enzymes.* Wisconsin, University Press.

Barker, H. A., 1936. The oxidation metabolism of the colorless alga, Prototheca zopfi. *J. Cell. Comp. Physiol.*, **8**: 231–250.

Barnes, M. R., 1944. The metabolism of the developing Rana pipiens as revealed by specific inhibitors. *J. Exp. Zool.*, **95**: 399–417.

Brachet, J., 1934. Étude du métabolisme de l'oeuf de grenouille (Rana fusca) au cours du developpement. I. La respiration et la glycolyse, de la segmentation a l'éclosion. *Arch. de Biol.*, **45**: 611–727.

Clifton, C. E., 1937. On the possibility of preventing assimilation in respiring cells. *Enzymologia*, **4**: 246–253.

Clifton, C. E., and W. A. Logan, 1939. On the relation between assimilation and respiration in suspensions and in cultures of Escherichia coli. *J. Bact.*, **37**: 323–540.

Giesberger, G., 1936. Beiträge zur Kentniss der Battung Spirillum Ehbg. Dissertation, Utrecht.

Holtfreter, J., 1943. Properties and functions of the surface coat in amphibian embryos. *J. Exp. Zool.*, **93**: 251–323.

Keilin, D., 1933. Cytochrome and intracellular respiratory enzymes. *Eng. Enzymforschg.*, **2**: 239–271.

Keilin, D., 1936. The action of sodium azide on cellular respiration and on some catalytic oxidation reactions. *Proc. Roy. Soc., London, B*, **121**: 165–173.

Korr, I. M., 1941. The relation between cellular metabolism and physiological activity. *Am. J. Physiol.*, **133**: 167.

Loeb, J., 1895. Untersuchungen über die physiologischen Wirkungen des Sauerstoffmangels. *Pflüger's Arch.*, **62**: 249–295.

Meyerhof, O., 1945. The origin of the reaction of Harden and Young in cell-free alcoholic fermentation. *J. Biol. Chem.*, **157**: 105–119.

Moog, F., and S. Spiegelman, 1942. Effects of some respiratory inhibitors on respiration and reconstitution in Tubularia. *Proc. Soc. Exp. Biol. and Med.*, **49**: 392–395.

Philips, F. A., 1940. Oxygen consumption and its inhibition in the development of Fundulus and various pelagic fish eggs. *Biol. Bull.*, **78**: 256–274.

Pollister, A. E., and J. A. Moore, 1937. Tables for the normal development of Rana sylvatica. *Anat. Rec.*, **68**: 489–496.

Ryan, F. J., 1941. Temperature changes and the subsequent rate of development. *J. Exp. Zool.*, **88**: 25–54.

Spiegelman, S., 1945. The effect of cyanide and azide on adaptive enzyme formation. Unpublished.

Spiegelman, S., and F. Moog, 1944. On the interpretation of rates of regeneration in Tubularia and the significance of the independence of mass and time. *Biol. Bull.*, **87**: 227–241.

Spiegelman, S., and H. B. Steinbach, 1945. Substrate-enzyme orientation during embryonic development. *Biol. Bull.*, **88**: 254–268.

Stannard, J. N., 1939. Separation of the resting and activity oxygen consumption of frog muscle by means of sodium azide. *Am. J. Physiol.*, **126**: 196–213.

Winzler, R. J., 1940. The oxidation and assimilation of acetate by baker's yeast. *J. Cell. Comp. Physiol.*, **15**: 343–354.

Winzler, R. J., 1943. A comparative study of the effects of cyanide, azide, and carbon monoxide on the respiration of baker's yeast. *J. Cell. Comp. Physiol.*, **21**: 229–252.

Winzler, R. J., 1944. Azide inhibition of anaerobic assimilation of glucose by yeast and its application to the determination of fermentable sugar. *Science*, **99**: 327–328.

Winzler, R. J., D. Burk, and V. du Vigneaud, 1944. Biotin in fermentation, respiration, growth, and nitrogen assimilation in yeast. *Arch. Biochem.*, **5**: 25–47.

Vol. 89, No. 2 October, 1945

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

STUDIES ON THE BIOCHEMISTRY OF TETRAHYMENA. IV. AMINO ACIDS AND THEIR RELATION TO THE BIOSYNTHESIS OF THIAMINE

GEORGE W. KIDDER AND VIRGINIA C. DEWEY[1]

Arnold Biological Laboratory, Brown University,
Providence, Rhode Island

It was reported earlier (Kidder and Dewey, 1942) that two species of Tetrahymena were able to carry out the synthesis of thiamine, if provided with a substance found mainly in the leaves of plants. This substance was called Factor S and was found in highest concentration in alfalfa leaf meal but could not be demonstrated from materials of animal origin. Factor S was characterized by its solubility in water and alcohol (up to 75 per cent), insolubility in ether and acetone, stability to prolonged heat in the presence of either alkali or acid, and its stability to ultraviolet radiation. It was shown to be dialyzable through cellophane and not to be precipitated by the salts of heavy metals. It was shown that Tetrahymena gave optimal growth in a medium consisting of "vitamin-free" casein, salts and a heat- and alkali-treated water extract of alfalfa meal. Very little growth occurred in the absence of the alfalfa extract and the addition of thiamine, riboflavin, pyridoxine, pantothenic acid, nicotinic acid, pimelic acid, *i*-inosital, uracil, or *p*-aminobenzoic acid either singly or in combination had no significant effect. Inasmuch as the heat- and alkali-treated alfalfa extract was certainly free of thiamine it was concluded that Tetrahymena could synthesize the thiamine required for its metabolic needs when supplied with Factor S. It was suggested that Factor S possibly acted as a catalyst necessary for the synthesis of the thiamine molecule.

It was recognized that the alfalfa extract used contained Factors I and II (Dewey, 1941; 1944) and we now know that the casein base contained Factor III (Kidder and Dewey, 1945a).

This work was criticized by Hall and Cosgrove (1944) on the basis that the "vitamin-free" casein used for the base medium was not free of thiamine. They reported growth of their strain of Tetrahymena in heat- and alkali-treated casein in the presence of thiamine and not in its absence. This criticism was shown to be invalid (Kidder and Dewey, 1944) when an extension of the earlier studies was carried out, using heat- and alkali-treated base media (casein, casein hydrolysate, gelatin, gelatin hydrolysate). It was then found that heat and alkali treatment of whole casein produced toxic substances which could not be overcome by thiamine addition for *T. geleii* W but could to a slight extent for *T. geleii* H. In tryptophane-supplemented gelatin (Harris), however, indefinitely transplantable growth was possible after all of the thiamine had been destroyed. The addition of thiamine did not affect the generation time but did increase significantly the maximum yield and survival. The addition of heat- and alkali-treated alfalfa extract decreased the generation time and raised the maximum yield to optimal for the species, and the addition of thiamine had no significant effect. This was interpreted as meaning that gelatin possessed low concentrations of Factors I, II, and S and that the final cessation of growth was due to the depletion principally of Factor S, as the addition of thiamine did raise the maximum yield.

---

[1] Aided by grants from the Morgan Edwards Fellowship Fund, the Manufacturers Research Fund for Bacteriology and Protozoology of Brown University.

One of the difficulties encountered in the earlier work was the separation of Factor S from Factors I and II. The heat and alkali treatment of peptones seemed to destroy the Factor I activity, but toxic substances were produced which rendered the medium inferior for our tests. Nevertheless, it was possible to show that lead acetate precipitate (containing no factor S) from plant material could replace the heat- and alkali-destroyed fraction only if thiamine was added. This was taken to mean that peptone contained no Factor S but did contain Factor II which was stable to the treatment used for dethiaminization, and Factor I which was unstable. It was recognized that little more could be done until active preparations of Factors I II could be obtained which were essentially free of both Factor S and toxic materials.

Recently we have been able to obtain such a preparation and it has been possible to test the activity of Factor S. This work, to be reported here, while confirming our earlier conclusions on thiamine synthesis, has forced us to alter our original theory concerning the role of Factor S in the metabolic activities of Tetrahymena.

Material and Methods

The organism used in the present study was the ciliated protozoan *Tetrahymena geleii* W, which is the strain used in the previous studies on thiamine synthesis (Kidder and Dewey, 1942; 1944). All work was done with pure (bacteria-free) cultures. The ciliates were grown in 2 ml. quantities of media in Pyrex tubes according to the technique described elsewhere (Kidder and Dewey, 1945b). All media, made with water twice distilled over permanganate in an all-Pyrex still, were adjusted to give a final pH of 6.8–7.0 and sterilization was by autoclaving. Serial transplants were made and results are recorded only after the third transplant. Transplants were made at 72 hour intervals using a bacteriological loop delivering approximately 0.008 ml. of fluid. Incubation was at 25° C. Population densities were determined by the direct counting technique (Kidder, 1941). All glassware used in this investigation was made chemically clean with sulfuric-dichromate solution, thoroughly rinsed and air dried before use.

In order to eliminate the possibility of cotton fibers contributing substances to the medium, Pyrex wool plugs were used extensively. It was found helpful to flame the protruding ends of the plugs until a thin crust had formed to eliminate the annoying strands inevitably present in this type of plug. This treatment fuses enough of the Pyrex strands to cause the plugs to hold their shape and increases appreciably the ease with which they may be handled.

Two types of base media were used for most of this work. One was 0.5 per cent hydrolyzed Eastman purified calfskin gelatin (Lot no. *144*). This hydrolysate was prepared by refluxing 100 gr. of gelatin in one liter of 25 per cent H_2SO_4 for 5 hours, removing the sulfate as $BaSO_4$ and reducing to the required concentration. Hydrolysate prepared with HCl was also used and the two were similar in every way. The gelatin hydrolysate was supplemented in all cases with 0.01 per cent *l*(−)-tryptophane and (with one exception to be noted later) with 0.02 per cent *dl*-valine. This base medium will be referred to as EGH.

The second type of base medium employed was a mixture of the eleven amino acids found to give optimum growth for this strain of *Tetrahymena geleii* (Kidder and Dewey, 1945a). These amino acids with the concentration in mg. per cent of each were as follows: *l*-(+)-arginine monohydrochloride—82; *l*(−)-histidine monohydrochloride—10; *dl*-isoleucine—35; *dl*-leucine—35; *dl*-lysine—60; *dl*-methionine—34; *dl*-phenylalanine—14; *dl*-serine—4; *dl*-threonine—20; *l*(−)-tryptophane—10; *dl*-valine—20. This base medium will be referred to as 11 AA. The sources of the amino acids used have been given elsewhere (Kidder and Dewey, 1945b).

Inasmuch as our primary concern was with thiamine all media were made up to contain other known growth factors, minerals and sugar to insure against limiting factors outside the scope of this investigation. Accordingly to our base media the following were always added:

	mg./ml.
Difco bacto dextrose	2.00
$MgSO_4 \cdot 7H_2O$	0.10
K_2HPO_4	0.10
$CaCl_2 \cdot 2H_2O$	0.05
$FeCl_3 \cdot 6H_2O$	0.00125
$MnCl_2 \cdot 4H_2O$	0.00005
$ZnCl_2$	0.00005
	Micrograms/ml.
Biotin methyl ester	0.00005
Calcium pantothenate	0.10
Nicotinamide	0.10
i-Inositol	1.00
Choline chloride	1.00
p-Aminobenzoic acid	0.10
Pyridoxine hydrochoride	0.10
Uracil	0.10
Folic acid[2]	0.01
Riboflavin	0.10

The sources of the salts and growth factors have been given earlier (Kidder and Dewey, 1945b).

Our preparation containing Factors I, II, and III was made from Liver Fraction L.[3] Fifteen grams of Liver Fraction L was dissolved in 750 ml. of distilled water, adjusted to pH 4.5, and extracted continuously for 96 hours in a liquid-liquid extracting apparatus (Wilson, Grauer, and Saier, 1940) with 750 ml. of *n*-butyl alcohol. The extracted material was freed of butyl alcohol, neutralized and

---

[2] Folic acid concentrate with a "potency" of 5000, furnished through the courtesy of Dr. R. J. Williams.

[3] Furnished through the courtesy of Dr. David Klein and the Wilson Laboratories.

LOGIC
OODS
LIBR

the volume reduced to 300 ml. This was designated 12L, and was found to contain adequate amounts of Factors I, II, and III. The pH of this preparation was adjusted to 9.5–10.5 with NaOH and heated in the autoclave at 123° C. for one hour for dethiaminization. This preparation will be designated 12L1, which was found to be free of Factor S activity. 12L1 was used as a supplement in a final concentration of 1 : 20.

Preparations containing Factor S were obtained from alfalfa meal. Water extract of alfalfa, as previously described (Kidder and Dewey, 1942; 1944), was heated in the autoclave at 123° C. for one hour at pH 9.5–10.5 to insure the destruction of thiamine. This preparation, designated A, was used in a final concentration of 1 : 10.

RESULTS

When Liver Fraction L is heated with alkali to destroy thiamine, changes take place which make it inferior as a source of supplementary factors for Tetrahymena. The addition of thiamine does not completely overcome these toxic effects, although the inhibition is less than that produced when proteose-peptone is dethiaminized. It was found, however, that toxic materials were not produced upon heating provided the Liver Fraction L was extracted previously with butanol. The butanol extraction was used originally for the removal of pyridoxin and riboflavin (to be reported in detail later).

TABLE I

Growth in EGH and 11 AA with and without added Factor S from dethiaminized alfalfa extract (A) and with and without added thiamine. All tubes contain 12L1 and the numbers represent organisms per ml. in the third serial transplant after 72 hrs. of growth.

Base	Additions			
	0	Thiamine	A	A + Thiamine
EGH	3,100	305,000	75,000	290,000
11AA	120,000	310,000	165,000	300,000

It was found that optimum growth resulted when a gelatin hydrolysate medium (with tryptophane, valine, and ten known growth factors), referred to as EGH, was supplied with 12L1 and thiamine, and very low growth occurred when the thiamine was omitted. The 12L1 was low in Factor S yet contained adequate amounts of Factors I, II, and III. This offered the opportunity to test the mode of action of Factor S, which could now be supplied from plant material without reference to the amounts of essential growth factors. Accordingly tests were set up using both EGH and 11 AA as base media, both supplemented with 12L1. To these base media were added various combinations of dethiaminized alfalfa extract (A) and thiamine. The results which were expected, namely the failure of growth unless either thiamine or Factor S was present, were not realized in 11 AA. Table I shows that very little growth occurred in the media based on EGH unless thiamine or Factor S was supplied but relatively good growth was obtained in the amino acid mixture in the absence of both. It will also be noted that thiamine is much more stimulatory, under these conditions, than is Factor S.

It was apparent from the foregoing results that the ability of Tetrahymena to synthesize thiamine was not dependent on the presence of Factor S when 11 AA was used as the base medium. This led to the conclusion that either some amino acid or combination of amino acids in the gelatin hydrolysate was blocking the synthetic mechanisms or that materials in the 12L1 were causing the block, the latter block being removed by some combination of the pure amino acids not present in the gelatin hydrolysate. The first of these possibilities was tested by making up an amino acid mixture based exactly on the published analysis for gelatin, but adding both tryptophane and valine (indispensable for this species). The ciliates behaved in this synthetic gelatin hydrolysate just as they had in 11 AA, so it was apparent that the first of the possibilities was untenable. The only known difference between the synthetic gelatin hydrolysate and EGH from a qualitative point of view was the inclusion in the former mixture of synthetic unnatural isomers (in the *dl* form, because of availability) of a number of the amino acids.

The addition of 11 AA to EGH plus 12L1 resulted in good growth without the addition of either thiamine or Factor S. This led us to test the effect of omitting each of the 11 amino acids singly from the 11 AA added to EGH. These results were inconclusive as fair growth occurred in all tubes. This was taken to mean that more than one of the 11 amino acids could counteract the inhibition to thiamine synthesis.

Table II

Growth of EGH with the addition of varying concentrations of racemic mixtures of amino acids. All tubes contain 12L1. The numbers represent organisms per ml. in the third serial transplant after 72 hrs. of growth.

Amino acid	Concentration of amino acid added (mg./ml.)					
	0.1	0.3	0.5	0.8	1.0	Control, nothing added
dl-phenylalanine	84,000	80,000	82,000	94,000	101,000	3,800
dl-methionine	92,000	8,600	8,000	6,000	7,800	
dl-serine	58,000	82,000	97,000	114,000	110,000	
dl-norleucine	31,000	11,500	0	0	0	
dl-aspartic acid	21,000	46,000	51,000	87,000	62,000	
dl-isoleucine	11,500	56,000	70,000	97,500	60,000	
dl-lysine monohydrochloride	4,500	15,000	58,000	61,000	78,000	
dl-threonine	6,000	31,000	42,000	66,000	81,000	
dl-homcystine	10,500	33,000	11,000	8,000	6,400	
dl-alanine	3,000	4,200	26,000	11,000	4,500	
dl-glutamic acid	6,500	7,500	12,500	21,000	37,000	

The next set of experiments was designed to determine whether or not the addition of single amino acids to the gelatin hydrolysate medium could counteract the inhibition to thiamine synthesis. Arbitrary amounts of each of nineteen amino acids were added to EGH. Thiamine synthesis occurred to a marked degree in some of the tubes, moderately in others and very little in some. In all cases where the inhibition was not removed the amino acid used was in its natural form while those amino acids which were most effective were synthetic.

This set of experiments was repeated using varying concentrations of the synthetic amino acids and some of the results are given in Table II. It will be seen that the effectiveness of the amino acids in releasing the inhibition of thiamine synthesis varied with the amino acid and the concentration. Phenylalanine was the most effective throughout the range of concentrations used while methionine was most effective in the lowest concentration. Norleucine was moderately effective at a concentration of 0.1 mg. per ml. but was toxic at 0.5 mg. per ml. or higher. These results indicated that the unnatural isomers were in some way able to release the inhibition of thiamine synthesis. It seemed more probable that the ratio between the two isomers was not the explanation, as some release of inhibition was found with some of the nonsynthetic amino acids. It is known that in the preparation of amino acids from natural sources some racemization is likely to occur and this might account for the small amount of activity.

This was shown to be the probable explanation by two sets of experiments. We had samples of natural *l*(+)-isoleucine, unnatural *l*(−)-isoleucine and synthetic *dl*-isoleucine. A comparison of the figures for *dl*-isoleucine in Table II with those in Table III shows that *l*(−)-isoleucine is effective in approximately one half the required concentration of *dl*-isoleucine. This is what is to be expected if only the unnatural isomer is effective in the removal of thiamine synthesis inhibition. The effectiveness of *l*(+)-isoleucine is low and increases with the concentration. This could be due to the occurrence of some racemization during its preparation.

TABLE III

Comparison of the effect of the natural isomer (l+) and the unnatural isomer (l−) of isoleucine, added to EGH + 12L1. Numbers represent organisms per ml. in the third serial transplant after 72 hrs. of growth.

Amino acid	Concentration of amino acid added (mg./ml.)					
	0.1	0.3	0.5	0.8	1.0	Control, nothing added
l(+)-isoleucine	7,800	4,100	6,300	9,800	11,500	3,100
l(−)-isoleucine	42,000	91,000	81,000	68,000	21,000	

TABLE IV

Comparison of the effect of the natural isomer (l−) and the racemic mixture (dl) leucine, added to EGH + 12L1. Numbers represent organisms per ml. in the third serial transplant after 72 hrs. of growth.

	Concentration of amino acid added (mg./ml.)					
	0.1	0.3	0.5	0.8	1.0	Control, nothing added
l(−)-leucine	2,500	3,500	10,500	14,000	13,500	3,800
dl-leucine	4,100	5,500	26,000	29,000	31,000	

When natural leucine was compared to *dl*-leucine the former was found to be less effective in the release of the synthesis inhibition (Table IV). The difference here, however, was not as marked, as the natural form appears to contain a considerable quantity of racemic mixture and the synthetic leucine is rather low in activity. It should be noted that we used Kahlbaum *dl*-leucine as this was found previously (Kidder and Dewey, 1945b) to be free of isoleucine, a common contaminant of many brands of synthetic leucine (Hegsted and Wardwell, 1944).

Inasmuch as EGH contained added *dl*-valine it was thought advisable to determine whether the unnatural isomer of this amino acid might be responsible for the ability of the ciliates to grow at all without added thiamine, Factor S or unnatural isomers of amino acids (see controls in Tables I–IV). Accordingly EGH minus valine was tested with varying concentrations of *dl*-valine with and without thiamine. Table V shows that without thiamine, very little growth occurs with no added valine, and that the inhibition to thiamine synthesis is counteracted more effectively the higher the concentration of added *dl*-valine. With added thiamine, however, the addition of valine had little effect. This indicates that the sample of gelatin used differs from our previous sample of Eastman de-ashed gelatin in that it contains nearly optimum amounts of natural valine for this species. It had previously been found (Kidder and Dewey, 1945b) that Eastman de-ashed gelatin would not support growth of *Tetrahymena geleii* W without added valine, even in the presence of thiamine. The fact that transplantable, though very low, growth occurs without the addition of any unnatural isomers of amino acids may mean that the inhibition to thiamine synthesis is never complete or that some racemization of the amino acids has occurred during hydrolysis.

Table V

Effect of the addition of dl-valine to EGH (minus valine). All tubes contain 12L1. Numbers represent organisms per ml. in the third serial transplant after 72 hrs. of growth.

	Concentration of dl-valine (mg./ml.)						
	0	0.05	0.1	0.3	0.5	0.8	1.0
Minus thiamine	150	2,500	4,000	7,500	16,000	37,000	45,000
Plus thiamine	190,000	210,000	265,000	310,000	305,000	325,000	315,000

When thiamine was added (0.1 micrograms per ml.) to any of the above described combinations, growth was always raised to approximately 300,000 ciliates per ml. Thiamine, therefore, although it can be synthesized by the ciliates, is very active as a stimulatory substance. It was of interest and importance to determine the amount of stimulation produced by different concentrations of thiamine when added to EGH plus 12L1; EGH plus 12L1 and one of the active amino acids; EGH plus 12L1 and Factor S; and 11 AA plus 12L1. Figures 1–4 show a summary of the activity of various concentrations of thiamine. The lowest concentration tested was 0.005 millimicrograms per ml. and in every case this amount gave significant stimulation. The stimulation was roughly proportionate to the concentration up to 0.001 micrograms per ml. In all cases, after this point, the amount of growth was increased more gradually but reached approximately the 300,000 level at 0.01 micrograms per ml. of thiamine when inhibition to thiamine synthesis was absent or removed. Ten times this amount of thiamine was required to raise

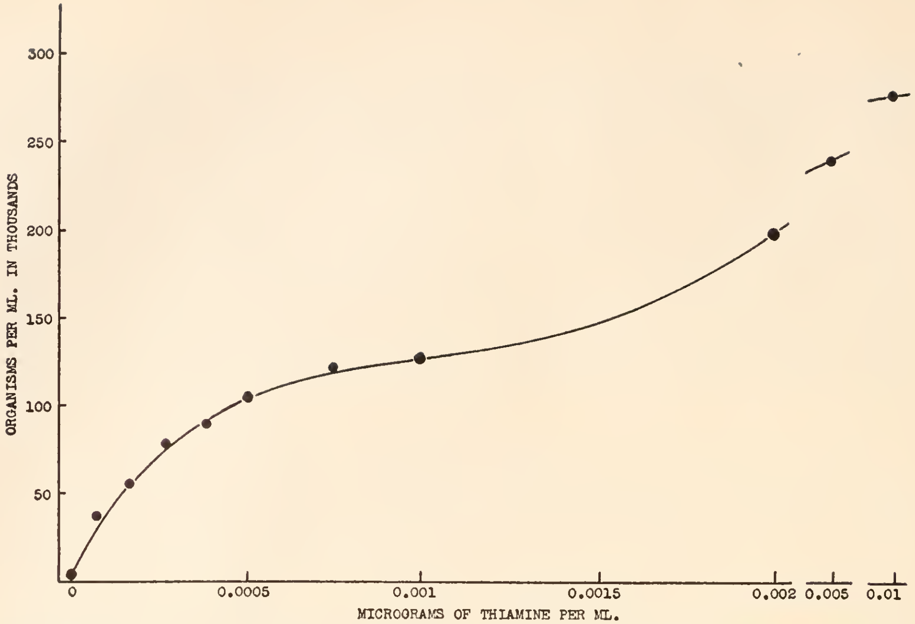


Figure 1. Curve of population densities at various concentrations of thiamine hydrocloride with gelatin hydrolysate (EGH) and dethiaminized butanol extracted Liver Fraction L (12L1) as base. The concentration of organisms was determined from the third transplant after 72 hrs. of growth.

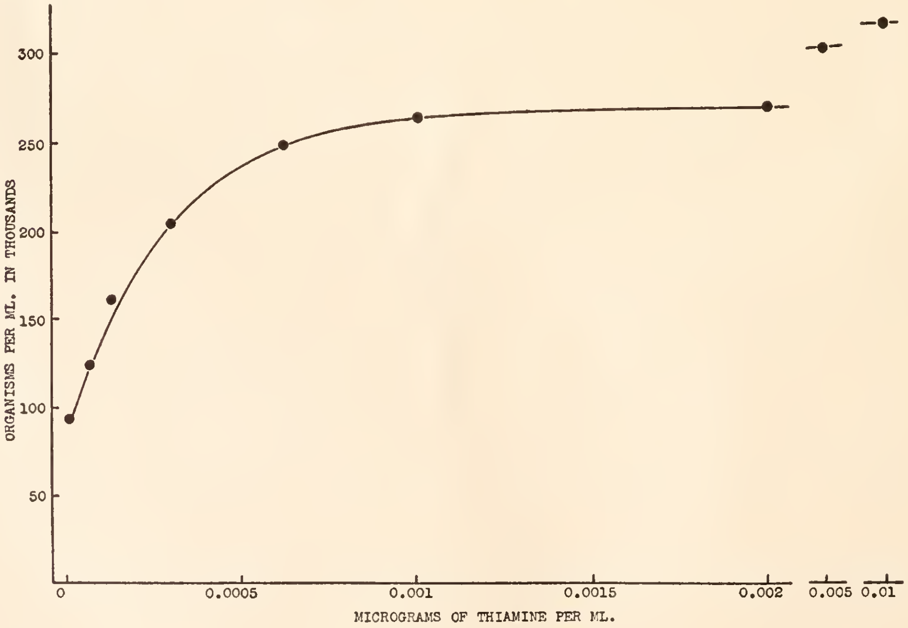


Figure 2. Curve of population densities at various concentrations of thiamine hydrochloride with EGH, 12L1 and *dl*-serine (0.5 mg./ml.) as base. Third transplant determinations after 72 hrs. of growth.

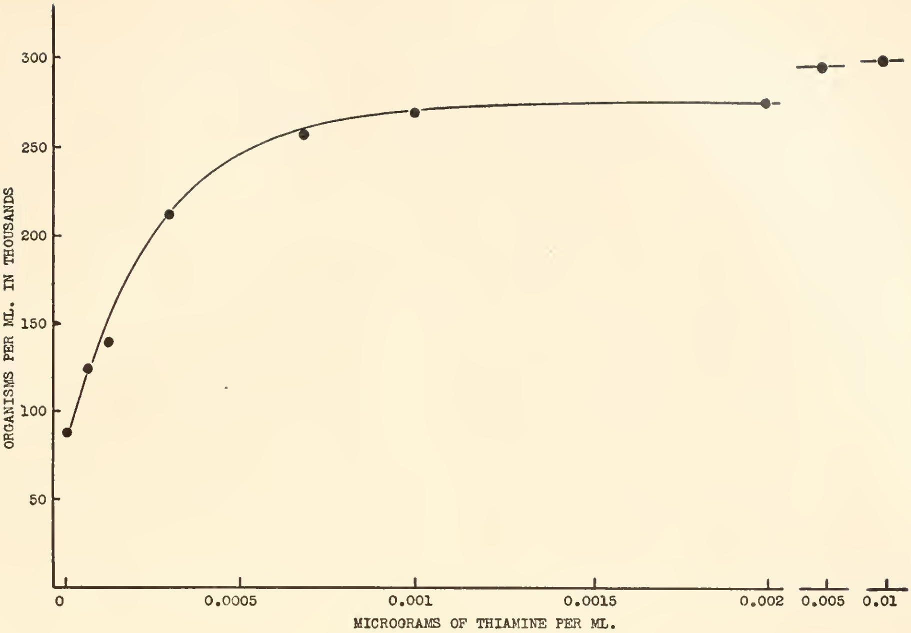


FIGURE 3. Curve of population densities at various concentrations of thiamine hydrochloride with EGH, 12L1 and dethiaminized alfalfa extract (A) as base. Third transplant determinations after 72 hrs. of growth.

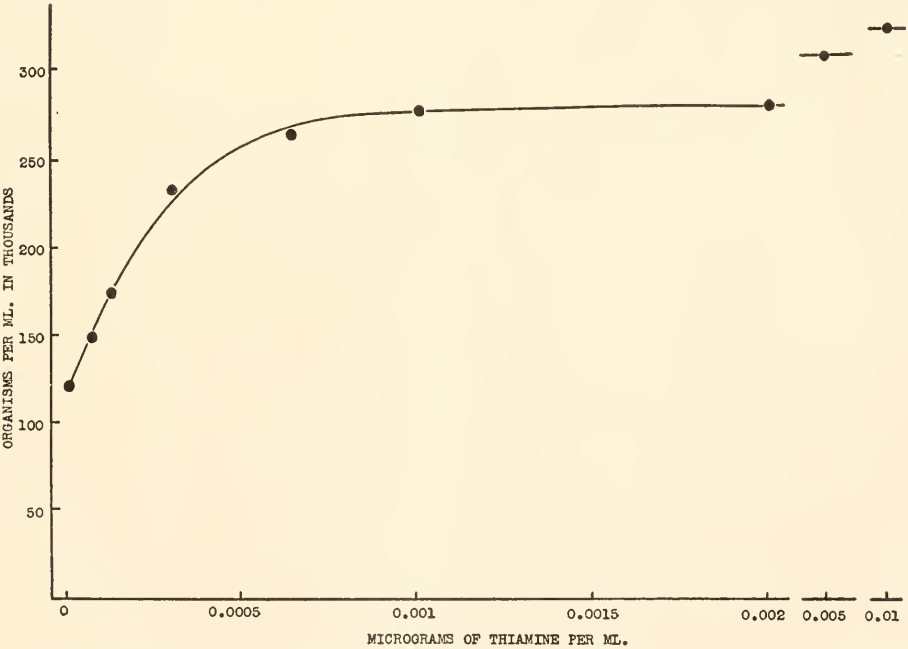


FIGURE 4. Curve of population densities at various concentrations of thiamine hydrochloride with the amino acid mixture (11 AA) and 12L1 as base. Third transplant determinations after 72 hrs. of growth.

the population to 300,000 per ml. where inhibition was pronounced (Fig. 1). These results show that Tetrahymena is far more sensitive to thiamine below a concentration of 0.001 micrograms per ml. than to higher concentrations.

An interesting and perhaps important point to be noted in the data shown in Figure 1 is the inflection which occurs in the curve above the 0.001 microgram per ml. level. The reasons for this inflection are not clear, although it seems possible that thiamine may be performing a double role where inhibition is pronounced. It may be supplying the vitamin needs of the organisms at the lower levels and acting to remove other inhibitions to growth as the concentrations increase.

Only the intact molecule of thiamine is capable of giving optimum stimulation. When the pyrimidine portion of thiamine (2-methyl-5-ethoxymethyl-6-amino pyrimidine) [4] or the thiazole portion (4-methyl-5-beta-hydroxyethyl thiazole) [4] were added separately or together some release of inhibition occurred. Table VI shows the results of these experiments. Both thiazole and pyrimidine produce stimulation in low concentrations but are mildly toxic at concentrations of 0.1 micrograms per ml. or higher. Thiazole and pyrimidine behave much the same as Factor S or the unnatural isomers of the amino acids, although to a less degree. They appear to cause the release of the thiamine synthesis inhibition and are themselves inhibitory in high concentrations.

TABLE VI

Growth in EGH plus 12L1 with varying concentrations of the thiazole and pyrimidine components of the thiamine molecule. Numbers represent organisms per ml. in the third serial transplant after 72 hrs. of growth.

	Concentration (micrograms/ml.)					
	0.001	0.005	0.01	0.1	0.5	Control, nothing added
Thiazole	3,000	8,400	20,000	1,400	1,000	2,900
Pyrimidine	1,200	5,300	17,500	3,200	2,600	
Thiazole and pyrimidine (total conc.)	2,500	11,000	24,000	2,400	2,500	

DISCUSSION

It appears from the foregoing results that there are substances present in natural materials which can block the synthetic mechanisms of Tetrahymena. Under the conditions of our experiments this blocking occurred specifically in the mechanism or mechanisms for the synthesis of the thiamine molecule. That this ciliate can synthesize thiamine, as was pointed out earlier (Kidder and Dewey, 1942; 1944) cannot be doubted, when the blocking substance is absent or the block is released. In our earlier work (Kidder and Dewey, 1942; 1944) where dethiaminized alfalfa extract was used as the supply of Factors I and II (Factor III was present in the casein and gelatin preparations; Kidder and Dewey, 1945a), it might be questioned whether the growth obtained in the absence of thiamine might be the result of no inhibitory substance rather than the presence of Factor S. However, it must be remembered that the addition of alfalfa extract to EGH plus 12L1 (which contains the inhibitory substance) removed the block. Whatever Factor S is, it is able to release the block to thiamine synthesis. But it is also seen that the unnatural isomers of the amino acids can act in a similar manner, so this reaction is far from specific as to counteracting substances. It was formerly proposed (Kidder and Dewey, 1942) that Factor S might act as a catalyst to the reaction wherein the thiamine molecule was synthesized. This hypothesis appears to be no longer tenable.

---

[4] Both the thiazole and the pyrimidine used were furnished through the courtesy of Dr. George W. Lewis and Merck and Co.

It does not seem likely that Factor S is, in reality, nothing more than racemic amino acids, for two reasons. If enough racemization occurred during the heat treatment of the alfalfa extract to account for the activity found then the same amount of racemization should have taken place in the heat treatment of 12L to produce 12L1. It was found, moreover, upon assaying the alfalfa extract for the indispensable amino acids for Tetrahymena that it did not contain enough of any one of the ten to support growth, when used in the concentration employed here. But a similar assay of 12L1 demonstrated almost optimum amounts of lysine; approximately half optimal amounts of arginine, threonine, and valine; and traces of histidine, isoleucine, leucine, and phenylalanine. It seems at present that Factor S represents some material present in alfalfa and the leaves of other plants (Kidder and Dewey, 1942), the activity of which is shared by the unnatural isomers of many of the amino acids.

The relation of amino acids to the ability of organisms to synthesize vitamins has been pointed out before. Snell and Guirard (1943) showed that alanine could replace pyridoxine for *Streptococcus fecalis* R (*S. lactis* R) and that alanine functioned to counteract the toxicity of glycine. It does seem strange, however, that the unnatural isomers appear to function in the release of thiamine synthesis inhibition for Tetrahymena. In nature this organism, being largely a bacteria feeder, probably would never be called upon to use its thiamine synthesis mechanism. The use of its ability to synthesize thiamine, therefore, is admittedly the result of artificial environmental conditions, as is also the very contact with the unnatural isomers of the amino acids.

It is apparent that, although Tetrahymena does possess the ability to synthesize thiamine, this vitamin is a potent stimulant to reproduction, size (Kidder and Dewey, 1944), and longevity (Johnson and Baker, 1943). Thiamine must, therefore, be included in complete media for this ciliate, but the amount needed appears to be less than has been previously used (Hall and Cosgrove, 1944; Kidder and Dewey, 1942; 1944).

It has been stated previously (Lwoff and Lwoff, 1938; Kidder and Dewey, 1942; 1944; Hall and Cosgrove, 1944) that heating peptones or proteins with alkali renders the media inferior for the growth of Tetrahymena. This condition could be partially counteracted for some strains by the addition of thiamine. The explanation appears now to rest in the partial destruction of serine, for we have found that if 11 AA is heat- and alkali-treated growth (with added 12L1) is very low but returns to normal with the addition of serine. Increased growth results with the addition of thiamine alone, however, indicating that this vitamin can replace serine. Or that serine (a dispensable but highly stimulatory amino acid in the presence of thiamine; Kidder and Dewey, 1945b), is one of the necessary factors for the synthesis of vitamin B_1.

The relationship which exists between the concentration of thiamine and the concentration of ciliates (Figures 1–4) might suggest that this organism would be useful for assay purposes. It would be difficult, however, to assay natural products for thiamine in a base medium composed of EGH plus 12L1, the only combination which gives a low blank, because of the likelihood of the introduction of Factor S or other materials of like nature with the substance to be assayed. Although we have not attempted to do this, it might be possible to arrange conditions so that 11 AA (Fig. 4) could be used and the values calculated as differences. Experiments directed to this end might prove valuable as the present microbiological methods are not entirely satisfactory. The majority of organisms used are stimulated by the thiamine components as well as by the whole molecule (Sarett and Cheldelin, 1944), require complex base media (Williams, 1942), or require many days of growth before results can be obtained (Robbins and Kavanagh, 1937).

SUMMARY

1. In Eastman gelatin hydrolysate (EGH) and Factors I, II, and III from Liver Fraction L (heat- and alkali-treated to destroy thiamine) the ciliate *Tetrahymena geleii* W grows very poorly without added thiamine.
2. A mixture of amino acids (11 AA) with the dethiaminized liver fraction supports fair growth without added thiamine.
3. There appear to be substances in the liver fraction or the gelatin hydrolysate or both which specifically block the mechanism for the biosynthesis of thiamine.
4. This block can be released by Factor S from alfalfa extract or by the unnatural isomers of a number of amino acids.
5. Some release of the inhibition to thiamine synthesis is produced by a few of the natural amino acids but this is probably due to the presence of low concentrations of unnatural isomers which result from racemization during preparation.
6. The unnatural isomer of isoleucine (the only unnatural isomer available for testing) was found to be active in approximately one half the concentration of the *dl*-isoleucine.
7. Thiamine is extremely stimulatory in low concentrations.
8. The thiazole and pyrimidine components are slightly stimulatory but this stimulation appears to be due to their ability to cause some release of the thiamine synthesis inhibition.

LITERATURE CITED

DEWEY, V. C., 1941. Nutrition of Tetrahymena geleii (Protozoa, Ciliata). *Proc. Soc. Exp. Biol. Med.,* **46**: 482–484.

DEWEY, V. C., 1944. Biochemical factors in the maximal growth of Tetrahymena. *Biol. Bull.,* **87**: 107–120.

HALL, R. P., AND W. B. COSGROVE, 1944. The question of the synthesis of thiamin by the ciliate, Glaucoma piriformis. *Biol. Bull.,* **86**: 31–40.

HEGSTED, D. M., AND E. D. WARDWELL, 1944. On the purity of synthetic *dl*-leucine. *Jour. Biol. Chem.,* **153**: 167–170.

JOHNSON, W. H., AND E. G. S. BAKER, 1943. Effects of certain B vitamins on populations of Tetrahymena geleii. *Physiol. Zool.,* **61**: 172–185.

KIDDER, G. W., 1941. Growth studies on ciliates. V. The acceleration and inhibition of ciliate growth in biologically conditioned medium. *Physiol. Zool.,* **14**: 209–226.

KIDDER, G. W., AND V. C. DEWEY, 1942. The biosynthesis of thiamine by normally athiaminogenic microorganisms. *Growth,* **6**: 405–418.

KIDDER, G. W., AND V. C. DEWEY, 1944. Thiamine and Tetrahymena. *Biol. Bull.,* **87**: 121–133.

KIDDER, G. W., AND V. C. DEWEY, 1945a. Studies on the biochemistry of Tetrahymena. II. Factor three. *Arch. Biochem.,* **6**: 433–437.

KIDDER, G. W., AND V. C. DEWEY, 1945b. Studies on the biochemistry of Tetrahymena. I. Amino acid requirements. *Arch. Biochem.,* **6**: 425–432.

LWOFF, A., AND M. LWOFF, 1938. La specificite de l'aneurine, facteur de croissance pour le cilie Glaucoma piriformis. *C. R. Soc. Biol.,* **127**: 1170–1172.

ROBBINS, W. J., AND F. KAVANAGH, 1937. Intermediates of vitamin B_1 and growth of Phycomyces. *Proc. Nat. Acad. Sci.,* **23**: 499–502.

SARETT, H. P., AND V. H. CHELEIN, 1944. The use of Lactobacillus fermentum 36 for thiamine assay. *Jour. Biol. Chem.,* **155**: 153–160.

SNELL, E. E., AND B. M. GUIRARD, 1943. Some interrelationships of pyridoxine, alanine and glycine in their effects on certain lactic acid bacteria. *Proc. Nat. Acad. Sci.,* **29**: 66–73.

WILLIAMS, R. J., 1942. Studies on the vitamin content of tissues II., Univ. Texas Publ. No. 4237: 7–13.

WILSON, D., R. C. GRAUER, AND E. SAIER, 1940. A simplified continuous extractor for estrogens and androgens. *Jour. Lab. Clin. Med.,* **26**: 581–585.

CERTAIN CHEMICAL FACTORS INFLUENCING ARTIFICIAL ACTIVATION OF NEREIS EGGS[1, 2]

PAUL G. LEFEVRE

Marine Biological Laboratory and Zoological Laboratory, University of Pennsylvania

Introduction

Stimulation must involve physicochemical changes within cells, and the nature of such changes has been the subject of much investigation, both experimental and speculative. The process of fertilization, and the closely related process of activation in artificial parthenogenesis, have attracted special attention; and evidence has been presented for a number of interesting interpretations of this type of activation. This report concerns a group of experiments indicating a peculiar relation of picric acid to the artificial activation of the eggs of *Nereis*. The proper interpretation of these experiments might contribute to the understanding of the stimulatory process. The experiments described developed from incidental observations in connection with heat-activation, during investigations concerned with the more general question of the mode of action of heat on protoplasm.

The peculiarities of heat-activation of the unfertilized *Nereis* egg were first described by Just (1915), who was able to interpret all his data in harmony with Lillie's "fertilizin" theories. In particular, Just attributed the gradual loss of sensitivity to heat, in eggs left standing in sea water, to the diffusion from them of some fertilizin-like substance, essential to the activating process. Heilbrunn (1925) took exception to this notion, in suggesting a "colloid chemical" interpretation of heat-parthenogenesis; he believed the decrease in sensitivity to heat might be due to the gradual loss of CO_2 from the medium, resulting in alkalinization of the intracellular fluid. Heilbrunn described three experiments in which the addition of 2–4 volumes per cent of n/10 HCl to old insensitive egg-suspensions restored their original sensitivity to heat.

To reveal a possible general relation between intracellular acidity or carbon dioxide concentration and the response of cells to increased temperatures, these three observations were extended. Heilbrunn's findings were in part confirmed; but with the accumulation of large numbers of experiments, considerable variation was encountered in the response of the heat-sensitivity of the eggs to increased CO_2 concentration through acidification of the sea water. Though such pronounced effects as described by Heilbrunn were often repeatable, as many batches of eggs seemed totally unresponsive to the same treatment. In the course of testing several organic acids in this connection, however, the anomalous properties of picric acid (2, 4, 6-trinitrophenol) came to light. Extension of these properties to processes of activation by means other than heat was then attempted.

---

[1] This study was carried out under the direction of Dr. L. V. Heilbrunn. I gratefully acknowledge his helpful suggestions throughout the investigations, and his valuable assistance in interpreting the results.

[2] A dissertation submitted to the faculty of the Department of Zoölogy of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Materials and Methods

Ripe females of the heteronereis form of *Nereis limbata* were captured between 8 and 10 p.m., as described by Lillie and Just (1913), and kept singly or in pairs in about 200 ml. of sea water in finger bowls overnight. These were kept cool either on a salt water bench in a stream of sea water, in a refrigerator, or in a room maintained at 15° C. The last was found by far the most satisfactory in maintaining the worms with eggs intact, with apparently no ill effects. A few worms shed their eggs during the night even at this reduced temperature; these were discarded.

All experiments were begun by the transfer of one or two *Nereis* to a Stender dish containing 25 ml. of sea water. The animals were cut transversely to release the eggs, and the carcasses were quickly removed. The eggs were then concentrated toward the center of the dish by gentle rotation. Either a small quantity of an especially dense suspension of eggs was then removed to another dish, or nearly all of the supernatant fluid was withdrawn by suction, and replaced by fresh sea water. All eggs were washed in this manner through at least another change of sea water, before use. In the earliest work, samples were always tested for normalcy by treatment with sperm from males caught in the same swarm. Only very rarely was any egg ever found which did not become normally fertilized, and all samples showed well over 99 per cent germinal vesicle breakdown. Since this dependability of *Nereis* eggs is well known, and since all danger of accidental contamination of eggs with sperm was to be avoided, no such tests were made in most of the later work. Experiments were always begun on the day following capture, so that the lapse of time between capture and the first treatment was never more than 20 hours, and was only rarely over 15 hours.

All transfers of eggs were made with ordinary medicine droppers. All treatments and exposures, unless otherwise indicated, were made in a volume of 25 ml.; the egg-suspensions were of such a density that, upon settling of the eggs to the bottom, no more than half, and usually much less, of the bottom of the container was covered with a single layer. Stender dishes of about 35 ml. capacity were used except for the exposures to high temperatures; the latter were carried out in 50 ml. beakers, in which the thermal insulation is much reduced. The beakers were immersed in a small deKhotinsky constant-temperature bath to a depth 2–3 mm. above the surface of the inside liquid. The temperature of the fluid within the beakers was brought to equilibrium (at slightly less than half a degree lower than the bath temperature) before the addition of 0.3–0.5 ml. of the egg-suspension. The activating temperature used varied between 33° and 35° C., as in Just's work (1915), but was held constant to within 0.1 of a degree for any single series of tests.

Since it soon became evident that the degree of stirring had a considerable effect on the response to heat, a standard policy in this regard was always followed: upon deposition of the eggs in the warm beakers, the pipette was filled and emptied ten times successively within 4–5 seconds. This was repeated 4 minutes after the beginning of the exposure; and the beaker was removed after 5 minutes of exposure, at which time a sample of 5–8 ml. was removed to a Syracuse watch glass. In some of the earlier work, the second stirring was performed at 15 minutes, the beakers removed at 20 minutes. This exposure, which is approximately Just's optimum, yielded a better percentage of swimmers, but the shorter exposure was found to produce the maximal amount of germinal vesicle breakdown, and was much more convenient in extended series of tests. A few tests indicated that further stirring and longer exposures led to no increase in the percentage of activation. A further trial showed that the immediate removal to Syracuse dishes was not essential; when the beakers were allowed to cool of their own accord, the residual heat did not affect the percentage of activation.

For counts of activation, 5–8 ml. of each egg-suspension were examined in a Syracuse watch glass at a magnification of about 100 ×. In certain cases involving a doubtful response, compression of the eggs between a slide and coverslip, as suggested by Heilbrunn and Wilbur (1937), and a higher magnification were necessary. The counts were made on the basis of the breakdown of the germinal vesicle, a reaction which normally occurs soon after fertilization. Counts were begun at a minimum of 2 hours after the application of the treatment in question. The advantages of the nuclear criterion are its rapidity of onset, its definite character (ordinarily admitting of easy and certain classification in counting), and its ready susceptibility to quantitative expression; the criterion is well established in work on artificial activation of this form. However, the fact should not be overlooked that the mere breakdown of the germinal vesicle in response to stimulation is seldom followed by development even approaching the normal, and there is rarely any cleavage at all. Various types of monsters are produced, mostly of the type described as due to "differentiation without cell-division," common in annelids. All of the types of stimulation used were capable of producing at least a small percentage of swimming forms, though seldom was anything like a normal trochophore seen. All counts were of 100 or 200 eggs selected by random movement of the watch glass on the stage of the microscope.

Results

Upon standing in sea water, almost all batches of eggs showed a gradual loss of sensitivity to heat, as described by Just (1915); a few, however, showed a very definite increase in sensitivity, after washing and long standing. This might perhaps be attributable to the washing away of inhibitors in the body fluids (Just, 1915); but the most pronounced of these exceptions was in a special batch in which the eggs stood in a deep layer at the bottom of a narrow container. Thus the responsible factor may have been the high CO_2 tension, in accordance with Heilbrunn's views (1925). Of several organic acids tested, however, only picric acid produced a consistent and pronounced reversal of this loss of sensitivity to heat. After a batch of eggs had become nearly or quite heat-insensitive, a bath of 15 minutes or more in sea water to which picric acid had been added to a concentration of about M/1000 (pH 6.6) was sufficient to elicit a significant response to the subsequent heat treatment in sea water. Yet the presence of the acid in the heat-treated suspensions completely prevented the activation of the eggs; if a response was to be obtained, the eggs had to be transferred back to sea water for the heat treatment.

These aspects of the action of picric acid were then tested in connection with activating agents other than heat. The agents used were ultra-violet irradiation (Heilbrunn and Wilbur, 1937), mixtures of sea water and isotonic (0.53 M) KCl (Wilbur, 1939), and mixtures of sea water and isotonic (0.35 M) sodium citrate

(Wilbur, 1941). Mixtures of KCl or citrate with sea water are denoted after the terminology of Wilbur (1941); thus a mixture of one volume of isotonic citrate and four volumes of sea water is called a "20 per cent sodium citrate mixture."

Experiments showing inhibition by picric acid of various types of activation

Heat—Of 15 experiments on the effect of picric acid on the sensitivity of eggs to heat, only one was inconsistent with the thesis that the acid inhibits the heat-activation. In these experiments, M/1000 picric acid was used, made up in sea water. Eight experiments proved useless, as the control percentages were too low to test any possible inhibition by the acid; the heat-sensitivity of these eggs is notoriously very variable between batches from different animals. The average of the seven experiments in which over 10 per cent of the control eggs responded is included in Table I, and shows a marked inhibition of the response by picric acid.

TABLE I

Inhibition by picric acid of activation of Nereis eggs by various agents

Activating agent	No. of expts.	Per cent activation in absence of picric acid	In picric acid, M/1000	
			Per cent activation	Per cent with incipient activation*
Heat	7	57	4	0
KCl mixtures	18	96	4	51
Sodium citrate mixtures	19	99	23	28

* As described on p. 147.

KCl mixtures—In fourteen experiments in which eggs were left indefinitely in a 25 per cent KCl mixture, and four similar experiments with a 50 per cent KCl mixture, almost always there was nearly 100 per cent activation in the absence of picric acid. When the acid was added to a concentration of M/1000, such activation occurred in only one instance; this case was distinctly unusual, as 74 per cent of the eggs were activated. Table I includes the averages for these experiments. However, in only 5 of the 18 tests was the breakdown of the germinal vesicle completely prevented. In the others, ordinary methods of observation (at 100 × magnification) did not reveal any certain change in appearance from the germinal vesicle stage, but a distinct nuclear outline could not be made out in many eggs. Compression of the eggs and higher magnification were necessary in counting these batches; the criterion employed was the visibility of a definite interface between the spherical nucleus and the cytoplasm. In the absence of this interface, the germinal vesicle was said to be broken down, even though no real alteration in the appearance of the egg was evident; the average percentage of the eggs so classified is presented in the last column of Table I. In these cells, the central nuclear region remained clear, the granular cortical opacity was retained, the oil droplets remained discrete and failed to migrate as in the activated eggs. None of the eggs of this type ever developed to a motile condition, or cleaved, or differentiated in any way. The appearance was as though nuclear breakdown had just barely begun when inhibition set in.

Sodium citrate mixtures—Complete or nearly complete inhibition of activation by picric acid was found in 12 of 19 experiments with citrate mixtures of 10–25 per cent. Of the other seven, two showed effective inhibition beyond the earliest stages of nuclear breakdown, as with the KCl mixtures (last column of Table I); one showed only moderate inhibition; only 4 of the 19 failed to show any significant inhibition. The averages are included in Table I. In these experiments, as in those with the KCl mixtures, the eggs were left in the activating agents indefinitely; counts were made with the eggs still in the various mixtures.

Ultra-violet irradiation—Only in relation to activation by ultra-violet rays did picric acid fail to exhibit an inhibitory effect. The presence of the acid (M/1000) in the sea water bathing the eggs did effectively prevent their activation by irradiation, but this action cannot be attributed to the effect of the acid on the eggs. Reduction of the depth of the egg-suspension to under 0.5 mm., so that the eggs are barely covered, permitted of ready activation by the rays, even in the presence of picric acid. The apparent inhibition in deeper samples is due to the absorption of the rays by the acid; the absorption spectrum of picric acid and picrates in salt solutions near neutrality (Eisenbrand and v. Halban, 1930; v. Halban and Litmanowitsch, 1941) is such that in any appreciable depth and concentration the supernatant fluid would prevent most of the active radiation from reaching the eggs, which always settle to the bottom of the dish. This interpretation is corroborated by the fact that a shield of picric acid in a quartz dish prevents any effect of ultraviolet rays on an underlying suspension of eggs in sea water.

Fertilization by sperm—Normal fertilization is completely inhibited in the solutions of acid used for the experiments above (in the range of M/1000). Addition of alkali to pH 8.0 did not affect this inhibition of fertilization. However, the removal of normally fertilized eggs to picric acid solutions within five minutes after fertilization (whether or not such solutions were alkalinized) did not appear to interfere with the normal development of the embryos; excellent survival and differentiation were obtained in the acid. Nevertheless, such embryos exhibited one outstanding anomaly: failure of the normal coalescence of the oil droplets. The oil in embryos growing in picric acid remained scattered as numerous discrete droplets; while under normal conditions these soon merge to form only a few, almost always four. The usual localization of the oil by migration (and segregation in cleavage) was not, however, altered in the course of development in picric acid solutions.

Experiments showing synergism between various activators and the removal from picric acid to ordinary sea water

Heat—Over 50 experiments tested the effect of baths in picric acid prior to exposure to heat in sea water. These showed a pronounced enhancement of the effects of the heat after the acid bath; not one showed a greater activation in the sample from sea water than in that from the acid. This relation between heat and removal from picric acid baths is shown in Figure 1. The synergistic action is evident only following the shorter baths, up to about 6 hours; since, after longer exposures to the acid, the mere removal to sea water was in itself sufficient to activate many eggs. The broken line curve in Figure 1 is made up from the combined data of all experiments involving removal of eggs from picric acid to sea water without further treatment. The other two curves on the same figure, however, cover data from paired samples of eggs, and compare the effects of heat on eggs previously bathed in picric acid (in sea water) and on eggs from the same source not so treated.

The synergistic action was evident over a wide range of concentration of picric acid: from 10^{-4} to just over 10^{-3} M. The effects increased with increasing concentration, but above M/1000 the results became less reliable, so that M/1000 was used regularly, and is the only concentration for which data are reported. It is evident from Figure 1 that the unfertilized eggs survived in the acid about twice as long as in sea water. Removal from sea water to the acid just prior to the expected onset of cytolysis (about 30 hours after removal from the animal) preserved the eggs as well as, but no better than, storage in the acid from the beginning.

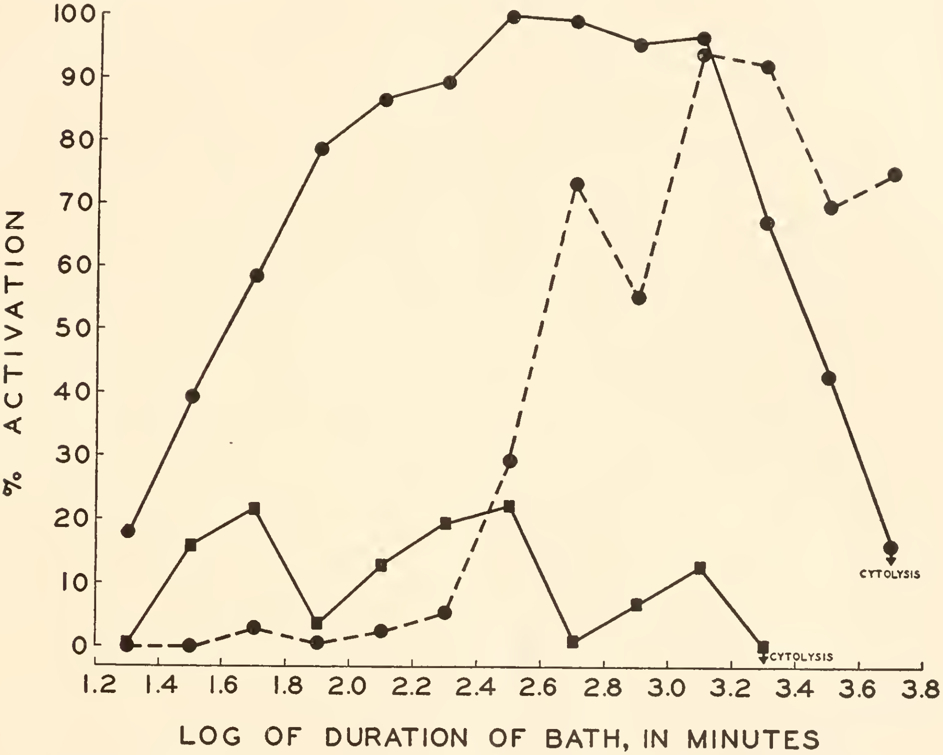


FIGURE 1. Relation of previous baths in picric acid to heat-activation of *Nereis* eggs.

Solid line connecting circular points—eggs heated after bath in M/1000 picric acid in sea water.

Solid line connecting square points—eggs heated after bath in sea water.

Broken line—eggs removed, unheated, from bath in M/1000 picric acid in sea water.

Each point is the average of all experiments performed in the logarithmic time interval denoted at the base-line. See text for further explanation.

KCl mixtures—The synergistic action of KCl mixtures and removal from picric acid to sea water was tested in 14 experiments, summarized in Figure 2(a). After 2–8 hours in the acid solutions, samples of eggs were removed to sea water and to 5 per cent KCl mixtures; a control sample of the same batch of eggs kept in sea water was simultaneously exposed to the 5 per cent KCl mixture. This concentration of KCl is just below that necessary to induce regularly an appreciable percentage of response in ordinary eggs. Though the combined treatment was not in every case sufficient to activate the eggs, most experiments showed a pronounced synergism, and none showed a difference in the opposite direction. The response of the eggs in these experiments, upon removal from picric acid baths to sea water, was somewhat less than average; thus the broken lines in the graphs in Figure 2 differ somewhat from the similar curve in Figure 1.

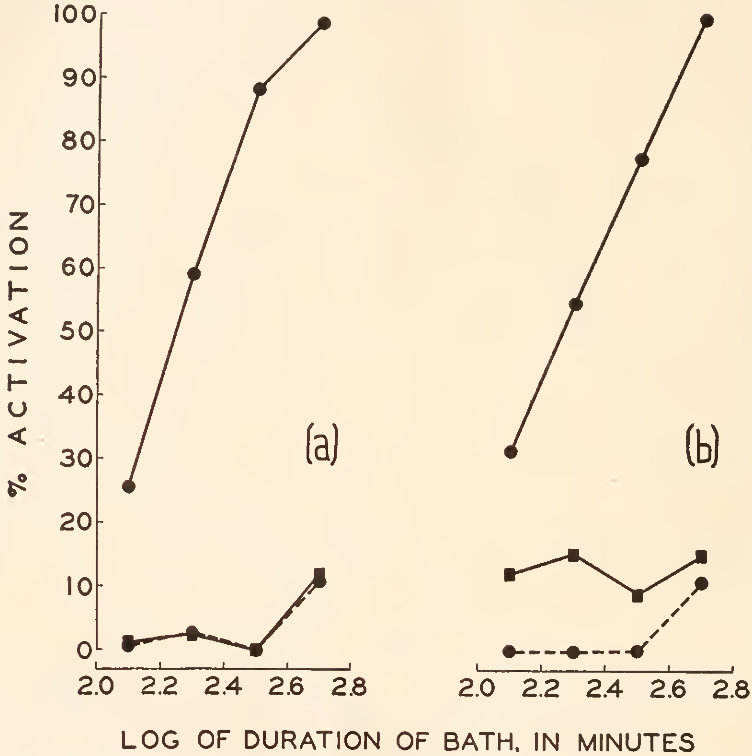


FIGURE 2. Relation of previous baths in picric acid to activation of *Nereis* eggs by (a) KCl mixtures, (b) sodium citrate mixtures.

Solid line connecting circular points—eggs treated after bath in M/1000 picric acid in sea water.

Solid line connecting square points—eggs treated after bath in sea water.

Broken line—eggs removed, untreated, from bath in M/1000 picric acid in sea water.

Each point is the average of all experiments performed in the logarithmic time interval denoted at the base-line. See text for further explanation.

Sodium citrate mixtures—The same action was demonstrated with 10 per cent sodium citrate in place of the 5 per cent KCl mixture; 14 of 19 experiments showed a decided synergism. Five failed to show any significant difference between control and experimental. These failures were all among the shorter exposures to the acid; the longer baths always resulted in increased sensitivity of the eggs to the citrate. This is illustrated clearly in Figure 2(b), which includes the data from all 19 experiments.

Ultra-violet irradiation—All attempts to show synergism between ultraviolet irradiation and removal from picric acid baths failed. A Uviarc mercury-vapor lamp, operating at 110 volts, 60 cycles, was used. The intensity of the radiation at the point at which the eggs were exposed was on the order of 6000 microwatts per square centimeter.[3] Under such conditions, no significant differences could be found between the response to irradiation of eggs just removed from picric acid baths and those from sea water.

Discussion

The data illustrate three aspects of the action of picric acid in relation to activation of the eggs:

(1) in the presence of certain concentrations of picric acid, heat-activation and chemical activation are prevented;

(2) removal from the same concentrations of picric acid to sea water, after a short stay in the acid, acts synergistically with other activating agents in causing nuclear breakdown;

(3) removal from the acid to sea water after longer stays in the acid leads to activation without assistance from other agents.

That fertilization and maturation of marine eggs is inhibited by acids is a common observation (Clowes and Greisheimer, 1920; Smith and Clowes, 1924; Tyler and Schultz, 1932; Tyler and Scheer, 1937); so that the inhibition by picric acid of artificial activation is not surprising. Similarly, the preservation of the unfertilized egg in picric acid against cytolysis and death is in accordance with many observations of this action of acids; some treatments were reported far more effective in this respect than picric acid appeared to be (Carter, 1931; Just, 1920; Smith and Clowes, 1924; Tyler and Horowitz, 1937a; Tyler and Dessel, 1939). The suggestion has even been made (Tyler, Ricci, and Horowitz, 1938) that the greater life-span of eggs in alcohol, dextrose, anoxic media, etc. (Gorham and Tower, 1902; Loeb, 1902; Loeb and Lewis, 1902; Lillie, 1931; Whitaker, 1937), can be explained in each case by the production of acids. The only odd aspect of the action of picric acid in this regard is that eggs stored in it for some time are subsequently oversensitive to stimulators, and eventually are activated merely by removal to sea water. This was observed after a stay in the acid of as much as 70 hours. This is entirely dissimilar to the acid activation of starfish eggs, as investigated extensively by Lillie (1926, 1927, 1934, 1941). Lillie's exposures were of only a few minutes' duration, and the eggs were visibly altered while in the acid; a slightly prolonged exposure destroyed the eggs altogether. In picric acid, however, the eggs remain apparently unchanged for days, but immediately respond when removed to sea water.

This fact leads to the postulate that picric acid may react with, or in some way inactivate, an activating agent produced within the egg. Above a certain concentration, this agent would lead to activation of the egg; in still greater concentration, or under other conditions, to cytolysis. This agent is apparently being constantly produced, and either diffuses from the egg, or is gradually destroyed as it is produced. But when picric acid is present within the egg, this agent is retained by the acid in an inactive form; when the egg is removed to sea water, the picric acid diffuses away, in turn releasing any acid bound with the activating agent. Thus the inhibition is removed, so that there is a sudden release of the accumulated activator within the egg, causing a response if the accumulation has been great enough.

---

[3] Thanks are due to Dr. A. C. Giese for this measurement.

Such a suggestion is in harmony with the synergism found between other activating agents and the removal from picric acid after exposures of lesser duration, and with the temporal pattern of the development of this synergism, as shown in Figures 1 and 2. The activating agents may be supposed to act by accelerating the production of the hypothetical activating substance; subliminal doses of these agents may then produce enough of the substance so that the added quantity released from the picric acid suffices to produce the response. That a still greater concentration may lead to cytolysis is indicated by the fact that less activation, with considerable cytolysis, is found when eggs are heated after a very prolonged exposure to picric acid, than when they are simply removed from the acid at the same time to sea water, without heating (Figure 1).

TABLE II

Synergism between various activating agents in stimulation of Nereis eggs

Activating agents		No. of expts.	Per cent activation		
A	B		A alone	B alone	Both
Heat	Sodium 5%	9	24	0	56
	citrate 10%	13	20	4	83
KCl 5%	Sodium 6%	1	0	0	86
	citrate 8%	1	0	2	99
	10%	1	0	78	100
Heat, without usual stirring	Stirring	4	15	0	39

The synergistic action indicates that at least to some extent activation is brought about through the same channels by all four agents: heat, KCl, sodium citrate, and removal from picric acid to ordinary sea water. Added evidence in this direction was obtained in experiments showing pronounced synergistic action between heat and citrate mixtures, and between KCl mixtures and citrate mixtures (Table II). As previously mentioned, stirring during exposure to heat had a pronounced enhancing action on the stimulatory effect of the heat, but stirring did not appear to act similarly in connection with the chemical activators. Mathews (1901) reported that Loeb and Fischer had been able to activate *Nereis* eggs by mechanical agitation alone, but all attempts in this direction failed.

Attempts to show synergism between ultra-violet irradiation and sodium citrate mixtures or removal from picric acid all failed; this is in keeping with the failure of picric acid to inhibit activation by ultra-violet rays. This may indicate that the radiation acts through a different mechanism than that involved in stimulation with the other agents. But under the conditions of the experiments the duration of the exposures to ultra-violet was on the order of 30–60 seconds, much less than with the other types of activation; this difference in the rate of activation may be the entire explanation for the non-conformance of the experiments with this type of activation.

Heilbrunn (1925), Heilbrunn and Wilbur (1937), and Wilbur (1939, 1941) have presented several lines of evidence indicating that the breakdown of the germinal vesicle in the *Nereis* egg involves a reaction of calcium ions with the colloids of the protoplasm, and an associated set of changes in viscosity. Heilbrunn proposed that stimulating agents act by freeing calcium ions from combination (with lipoprotein) in the cell cortex, so that the calcium may react with the inner protoplasm; this interpretation of stimulation has been applied not only to the eggs of *Nereis,* but to cells in general. If such a mechanism is actually involved in the response of the *Nereis* egg, it might be expected that a penetrating acid would inhibit activation. The picric acid might acidify the protoplasm to the extent that the amphoteric protein molecules would become predominantly cations, with less Ca-binding capacity than previously. This interpretation would perhaps also explain the activation found upon removal of eggs from picric acid baths to sea water; the calcium freed from the cortex by the acid could react with the cell interior upon removal of the acid. Thus the same agent would act, in a sense, both as activator and as anesthetic. A serious difficulty with this explanation of the data lies in the fact that the eggs must be left in the acid for several hours, if they are to respond upon removal to sea water. This would require the assumption that the liberation by the acid of calcium ions from the cortex is a very slow process; or else that the acid continues to accumulate within the egg over a period of hours, quickly rising to the inhibitory concentration, but only after hours attaining the concentration active on the cortex. Neither of these assumptions is impossible, but both are rather involved.

If the action of picric acid were due to this proposed effect on the Ca-binding properties of proteins, other acids might be expected to act similarly. The action of other acids similar to picric, as regards pK and penetrating ability, has not yet been investigated; however, acetic, boric, and tannic acids have been used in experiments similar to those performed with picric acid. Acetic acid was used in concentrations from M/6000 to M/300; boric acid, from $M/10^5$ to M/5; and tannic acid, from $M/10^6$ to M/100; the upper limits of concentrations used were factors of the solubility and the effects of the acids on the eggs. Over M/1000, acetic acid often injured the eggs irreversibly, so that they were not fertilizable; this makes it difficult to evaluate cases of inhibition by acetic acid of activation, in the absence of tests for reversal of the effect. Ten to twenty experiments were performed with each acid in attempts to demonstrate synergism with heat, in the manner of picric acid; the duration of the baths ranged from 30 minutes to 24 hours. On a few occasions, acetic acid in concentrations around M/500 (concentrations not always innocuous) showed the synergistic action, but as often acted in the opposite manner (probably because of injury to the eggs). On one batch of eggs, M/10–M/20 boric acid also showed some synergistic action with heat, but this did not recur in similar experiments with other batches of eggs.

A further corroboration of the interpretation in terms of an activator-substance was sought in several attempts to accumulate the activator more rapidly by heating the eggs in picric acid, with subsequent release to sea water. In only 3 of 16 such experiments was there markedly more activation in the eggs so treated than in those similarly exposed to the acid without application of the heat. However, none showed differences in the other direction; no data of any experiment thus far performed militates against the suggested scheme.

The completely reversible inhibitory action of picric acid is similar to the action of isotonic citrate in the experiments of Heilbrunn and Wilbur (1937) and Wilbur (1941). Since the citrate is presumed to act by removing calcium ions from solution by the formation of calcium citrate, there is a suggestion that perhaps calcium picrate is a similarly weakly dissociated salt. However, a few measurements of the electrical resistance of calcium picrate solutions showed that the equivalent conductance increased only slightly with dilution over the range n/100–n/10,000. (The increase was in proportion to that found with $CaCl_2$ in the same concentrations; the equivalent conductance of calcium citrate increased enormously with dilution over this range of concentration.) Thus the inhibitory action of picrate cannot be explained on the same basis as that applied to citrate inhibition.

In their extensive experiments on the peculiar action of many substituted phenols on the eggs of the sea-urchin, Clowes and Krahl (1936), Krahl and Clowes (1936, 1940), and Tyler and Horowitz (1937b, 1938) found picric acid one of only two or three inactive members of this group of compounds. Inhibition of cleavage was encountered only at concentrations around M/100 or more, and the stimulation to respiration characteristic of this chemical group was lacking altogether. The calculations of Tyler and Horowitz showed that the concentration of dissociated picrate inside the cells was about 100 × that at which the related substances showed similar effectiveness. The same sort of relation was found for the other relatively inactive phenols. The latter should be tested in experiments similar to those with picric acid reported here. Such investigations might aid in deciding whether the action of picric acid is to be attributed to its acidity or to its particular molecular configuration.

Perhaps picric acid is unique in its combination of a low pK and a rapid rate of penetration into cells. The other phenols found to be exceptional (as regards inhibition of cleavage and stimulation to respiration) may share this combination of properties. Additional experimentation involving alteration of the picrate/picric acid ratio in the solution (through addition of HCl or NaOH) is also suggested; such data might strongly indicate whether the acidity or the picrate itself is the active factor. On either basis, the present data clearly show that, while anesthetizing the eggs, this active factor constantly renders them increasingly sensitive to the removal of the anesthetization, and to subsequent stimuli. Proper interpretation of this fact might lead to a significant contribution to the understanding of the nature of stimulation.

SUMMARY

1. Germinal vesicle breakdown in *Nereis limbata* eggs, brought about by heat, or addition of KCl or sodium citrate to the sea water, was inhibited by the addition of picric acid at about M/1000.

2. After immersion for a few hours in M/1000 picric acid in sea water, germinal vesicle breakdown occurred upon application of subliminal doses of heat, KCl, or sodium citrate.

3. After immersion for 6–70 hours, removal of the eggs from picric acid to ordinary sea water caused germinal vesicle breakdown.

4. Activation by ultra-violet irradiation did not conform in these relations to picric acid, under the conditions of the experiments.

5. These results are interpreted on the basis of a hypothetical activating substance produced within the egg, and inactivated or bound by picric acid.

6. The relation of picric acid to the calcium ion and the combination of calcium with protoplasmic proteins is considered, in an alternative explanation of the results.

LITERATURE CITED

CARTER, G. S., 1931. Iodine compounds and fertilization. III. The fertilisable life of the eggs of Echinus esculentis and Echinus miliaris. *Jour. Exp. Biol.*, **8**: 194.

CLOWES, G. H. A., AND E. GREISHEIMER, 1920. Sperm as an inhibitant of maturation and fertilization. *Jour. Biol. Chem.*, **41**: li.

CLOWES, G. H. A., AND M. E. KRAHL, 1936. Studies on cell metabolism and cell division. I. On the relation between molecular structures, chemical properties, and biological activities of the nitrophenols. *Jour. Gen. Physiol.*, **20**: 145.

EISENBRAND, J., AND H. v. HALBAN, 1930. Über die Lichtabsorption der Nitrophenole. I. Schwach alkalische wässerige Lösungen. *Z. physik. Chem.*, A **146**: 30.

GORHAM, F. P., AND R. W. TOWER, 1902. Does potassium cyanide prolong the life of the unfertilized egg of the sea-urchin? *Amer. Jour. Physiol.*, **8**: 175.

v. HALBAN, H., AND M. LITMANOWITSCH, 1941. Über Ionenassoziation und Absorptionsspektrum. II. *Helv. Chim. Acta*, **24**: 38.

HEILBRUNN, L. V., 1925. Studies on artificial parthenogenesis. IV. Heat parthenogenesis. *Jour. Exp. Zool.*, **41**: 243.

HEILBRUNN, L. V., AND K. M. WILBUR, 1937. Stimulation and nuclear breakdown in the Nereis egg. *Biol. Bull.*, **73**: 557.

JUST, E. E., 1915. Initiation of development in Nereis. *Biol. Bull.*, **28**: 1.

JUST, E. E., 1920. The fertilization-reaction in Echinarachnius parma. IV. A further analysis of the nature of butyric acid activation. *Biol. Bull.*, **39**: 280.

KRAHL, M. E., AND G. H. A. CLOWES, 1936. Studies on cell metabolism and cell division. II. Stimulation of cellular oxidation and reversible inhibition of cell division by dihalo and trihalophenols. *Jour. Gen. Physiol.*, **20**: 173.

KRAHL, M. E., AND G. H. A. CLOWES, 1940. Studies on cell metabolism and cell division. IV. Combined action of substituted phenols, cyanide, carbon monoxide, and other respiratory inhibitors on respiration and cell division. *Jour. Gen. Physiol.*, **23**: 413.

LILLIE, F. R., AND E. E. JUST, 1913. Breeding habits of the heteronereis form of Nereis limbata at Woods Hole, Mass. *Biol. Bull.*, **24**: 147.

LILLIE, R. S., 1926. The activation of starfish eggs by acids. *Jour. Gen. Physiol.*, **8**: 339.

LILLIE, R. S., 1927. The activation of starfish eggs by acids. II. The action of substituted benzoic acids and of benzoic and salicylic acids as influenced by their salts. *Jour. Gen. Physiol.*, **10**: 703.

LILLIE, R. S., 1931. Influence of cyanide and lack of oxygen on the activation of starfish eggs by acid, heat and hypertonic sea-water. *Biol. Bull.*, **60**: 288.

LILLIE, R. S., 1934. The influence of hypertonic and hypotonic sea water on the artificial activation of starfish eggs. *Biol. Bull.*, **66**: 361.

LILLIE, R. S., 1941. Further experiments on artificial parthenogenesis in starfish eggs, with a review. *Physiol. Zool.*, **14**: 239.

LOEB, J., 1902. Maturation, natural death and the prolongation of the life of unfertilized starfish eggs (Asterias forbesii) and their significance for the theory of fertilization. *Biol. Bull.*, **3**: 295.

LOEB, J., AND W. H. LEWIS, 1902. On the prolongation of the life of the unfertilized eggs of sea-urchins by potassium cyanide. *Amer. Jour. Physiol.*, **6**: 305.

MATHEWS, A. P., 1901. Artificial parthenogenesis produced by mechanical agitation. *Amer. Jour. Physiol.*, **6**: 142.

SMITH, H. W., AND G. H. A. CLOWES, 1924. The influence of hydrogen ion concentration on unfertilized Arbacia, Asterias, and Chaetopterus eggs. *Biol. Bull.*, **47**: 304.

SMITH, H. W., AND G. H. A. CLOWES, 1924. The influence of hydrogen ion concentration on the development of normally fertilized Arbacia and Asterias eggs. *Biol. Bull.*, **47**: 323.

SMITH, H. W., AND G. H. A. CLOWES, 1924. The influence of hydrogen ion concentration on the fertilization process in Arbacia, Asterias, and Chaetopterus eggs. *Biol. Bull.,* **47**: 333.

TYLER, A., AND F. W. DESSEL, 1939. Increasing the life span of unfertilized Urechis eggs by acid. *Jour. Exp. Zool.,* **81**: 459.

TYLER, A., AND N. H. HOROWITZ, 1937a. The molecular species concerned in the action of substituted phenols on marine eggs. *Biol. Bull.,* **73**: 377.

TYLER, A., AND N. H. HOROWITZ, 1937b. . The action of certain substituted phenols on marine eggs in relation to their dissociation. *Proc. Nat. Acad. Sci.,* **23**: 369.

TYLER, A., AND N. H. HOROWITZ, 1938. The activities of various substituted phenols in stimulating the respiration of sea urchin eggs. *Biol. Bull.,* **75**: 209.

TYLER, A., N. RICCI, AND N. H. HOROWITZ, 1938. The respiration and fertilizable life of Arbacia eggs under sterile and nonsterile conditions. *Jour. Exp. Zool.,* **79**: 129.

TYLER, A., AND B. T. SCHEER, 1937. Inhibition of fertilization in eggs of marine animals by means of acid. *Jour. Exp. Zool.,* **75**: 179.

TYLER, A., AND J. SCHULTZ, 1932. Inhibition and reversal of fertilization in eggs of the echinoid worm, Urechis caupo. *Jour. Exp. Zool.,* **63**: 509.

WHITAKER, D. M., 1937. Extension of the fertilizable life of unfertilized Urechis eggs by alcohol and by dextrose. *Jour. Exp. Zool.,* **75**: 155.

WILBUR, K. M., 1939. The relation of the magnesium ion to ultra-violet stimulation in the Nereis egg. *Physiol. Zool.,* **12**: 102.

WILBUR, K. M., 1941. The stimulating action of citrates and oxalates on the Nereis egg. *Physiol. Zool.,* **14**: 84.

A STUDY OF THE GOLGI APPARATUS IN CHICKEN GIZZARD EPITHELIUM BY MEANS OF THE QUARTZ MICROSCOPE

HOPE HIBBARD AND GEORGE I. LAVIN

Oberlin College, Oberlin, Ohio, and The Rockefeller Institute for Medical Research, New York City

The only fact about the Golgi apparatus that is universally accepted is that it is a cytoplasmic constituent of most cells which, after special fixation, blackens with silver nitrate or osmic acid. Controversies as to its structure, composition, function, and even its existence in the living cell, have been carried on continuously ever since Golgi originally described such a cellular constituent in 1898, totaling considerably over 2000 published papers. Mere descriptions of the blackened apparatus appear to be no longer fruitful. Furthermore, since cells must differ in order to carry on their specialized functions, warnings have been voiced against making hasty generalizations about all cells from studies on particular cells. The work reported in this paper pretends to nothing further than an analysis of certain features in one type of cell.

The Golgi apparatus in the lining of the chicken gizzard near its junction with the proventriculus is unusually spectacular and easily demonstrated. It can practically always be clearly shown after the usual osmic acid or silver nitrate techniques. Moreover, its size is enormous (Fig. 5). For these reasons chicken gizzard material is excellent for the study of the nature of the peculiar region of the cytoplasm where this network appears.

Previous studies have followed its changes in form during embryonic development of the gizzard (Hibbard, 1942). It can be demonstrated only in fixed material after appropriate impregnations. It can never be seen in living cells or in cells otherwise well fixed but not fixed by the usual methods for the Golgi apparatus. The only cytoplasmic inclusions which are rendered visible by methods other than silver and osmic impregnations in the general zone occupied by the Golgi apparatus, are vacuoles which may be stained vitally or postvitally with neutral red, and occasional filamentous mitocondria. Hibbard (1942) has suggested that these vacuoles might be the antecedents of the typical Golgi network.

In an interesting series of papers, Worley (1943, 1944) has pointed out the high susceptibility of cytoplasmic inclusions, in many types of cells, to displacement or to changes in form and appearance with very slight changes in salt concentration in the surrounding fluid. Within ten seconds such distortions may take place so that quite different bodies from the original ones may be formed. Worley suggests, as Parat did nearly twenty years ago, that the fixed picture as it appears in sections may not at all resemble the living conditions.

Analysis of the Golgi apparatus by special types of illumination is not new. Monné in 1939 published two papers dealing with the appearance of the Golgi apparatus in Helix spermatocytes, using polarized light in one case and dark field illumination in the other, in order to demonstrate physical characteristics of difference between the Golgi bodies and other cytoplasmic constituents. It must be remembered that his results apply to spermatocytes only and great care should be taken not to generalize them to apply to all Golgi bodies unless similar observations are made on other types of cells. There is a great difference between the spermatocyte Golgi apparatus and that in the glandular cells described in this paper, in staining reactions, susceptibility to deformation and the variety of methods by which it may be seen at all (Hibbard, 1945).

The present study was undertaken to determine, by means of ultraviolet microphotography, something about the nature of the cytoplasmic zone which becomes a complex network of blackened material after impregnation. It is not a study of the living cell, and may therefore be the analysis of an artifact. But if so it is one of extremely uniform occurrence in the chicken gizzard and one which undoubtedly has some precursor substance or some physical state of the material in the living cell which produces the localized black network in the fixed sections in perfectly regular fashion.

The technique employed was to make 5 μ sections, either in paraffin or frozen, of material fixed in 7 per cent neutral formalin or in Da Fano, one of the fixatives nearly always successful in demonstrating the Golgi apparatus in gizzard epithelium. These sections were mounted on quartz slides under quartz coverslips and photographed with the quartz microscope using the 2537 Å line of mercury as the light source. The sections were unstained. Lavin (1943) has described the technique of ultraviolet microphotography.

Examination of the microphotographs shows that most cells have no peculiar tone variation in the zone in question (Fig. 3). However, in those cells with any apparent difference in the Golgi zone as compared with the rest of the cytoplasm, the Golgi zone appears somewhat paler (Figs. 1, 2, and 4).

It is known that nucleic acid has an absorption maximum of 2600 Å and proteins which contain tyrosine and tryptophane have an absorption maximum at 2800 Å. Nucleoproteins will have a maximum at some point intermediate. In order to show that these materials absorb in the ultraviolet region of the spectrum while in the solid state, microphotographs of globulin and of nucleic acid pellets were taken with the quartz microscope. They were cut and treated as if they were blocks of tissue. These photographs are reproduced in Figure 7 and Figure 8. It will be noted that while the nucleic acid is dark, the globulin remains pale. In a similar way microphotographs taken in the ultraviolet should demonstrate in tissues, with-

---

FIGURE 1. Microphotograph, ultraviolet illumination; 5 μ paraffin section after Da Fano fixation; no stain.

FIGURE 2. Microphotograph, ultraviolet illumination; 5 μ paraffin section after 7 per cent formalin fixation; no stain.

FIGURE 3. Microphotograph, ultraviolet illumination; 5 μ frozen section after 7 per cent formalin fixation; no stain.

FIGURE 4. Microphotograph, ultraviolet illumination; 5 μ paraffin section after 7 per cent formalin fixation; no stain.

FIGURE 5. Microphotograph, visible light with green filter; 5 μ paraffin section after Da Fano fixation followed by silver nitrate impregnation and reduction; no further stain.

FIGURE 6. Same as Figure 5.

FIGURE 7. Microphotograph, ultraviolet illumination; section of a pellet of globulin.

FIGURE 8. Microphotograph, ultraviolet illumination; section of a pellet of nucleic acid.

(In all figures, G—Golgi zone and N—nuclear region.)

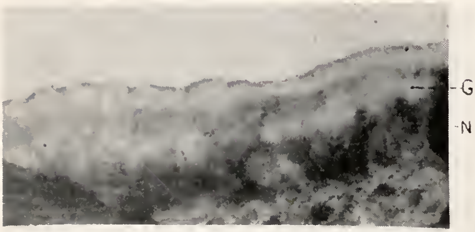


Fig 1

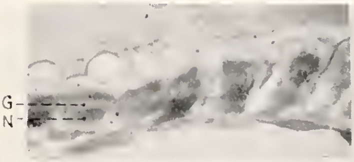


Fig 2

Fig. 3

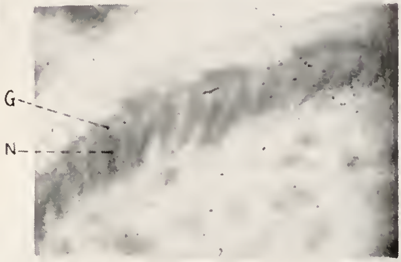


Fig 4

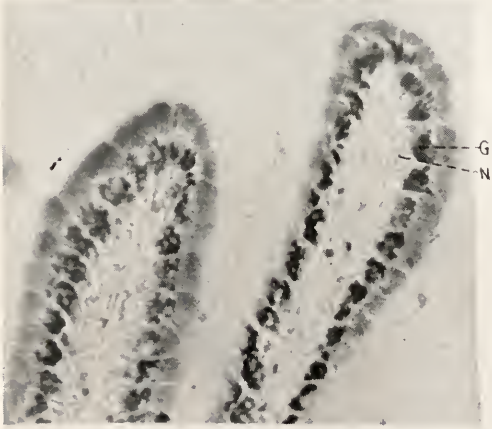


Fig. 5

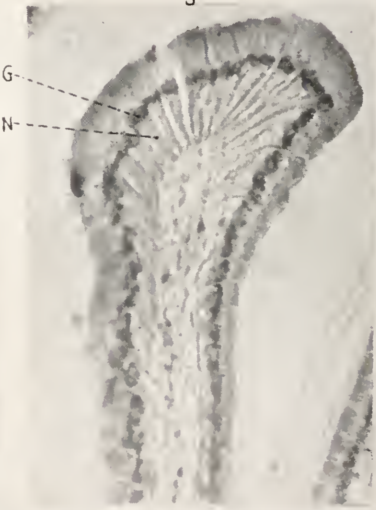


Fig. 6

Fig. 7

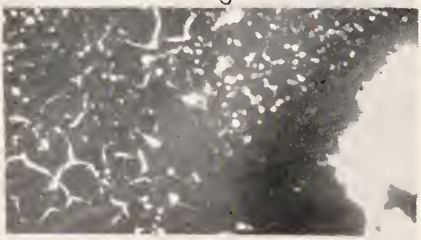


Fig. 8

Figures 1–8

out the necessity of staining, the presence of any substance of appropriate absorption maximum. Since the absorptive capacities of many organic tissue components are known and thus this method of demonstrating cell inclusions is a reflection of their chemical composition, we may say that the photographs present evidence that the Golgi zone does not contain appreciable amounts of nucleoproteins, nucleic acids or proteins containing tryptophane and tyrosine. The position of the Golgi apparatus in most cells is characteristically in close proximity to the nuclear surface, a position of possible physiological importance. This evidence that the region does not contain nucleoproteins or nucleic acid in large amounts if at all, is of some importance, particularly in view of the fact that most histological stains are in no sense chemical tests.

Our results also shed some light on the further question, which is of some interest: why does special fixation have to be practised before the subsequent impregnation will "take?" Examination of Figures 1, 2, and 4 will show that the Golgi zone is paler than the rest of the cytoplasm in many of the cells. In all probability this clear zone corresponds to the area blackened by silver nitrate. The curious fact is that this paler area may appear after formalin fixation whether the sections are imbedded in paraffin or frozen (Figures 2, 3, and 4), and also after Da Fano fixation without subsequent silver impregnation (Fig. 1). Silver impregnation applied after Da Fano fixation will produce a spectacular type of Golgi apparatus as shown in Figures 5 and 6, while exactly similar impregnation after neutral formalin as the fixative will produce only miscellaneous black granules throughout the cell with no greater blackening of the Golgi zone than of other regions. This seems to indicate that the Da Fano fixative either preserves some constituent lost in the formalin, or else adds itself to material already there, to make it reduce the silver in the conspicuous network. The identical absorption capacities of the region to ultraviolet light, whether the fixative be formalin or Da Fano, suggests a similarity in the quality of the fixed protoplasm. Why the Da Fano fixative should create a focal point exactly in the Golgi zone for the subsequent reduction of the silver, is not so clear. It may be that the Golgi zone is the site of aqueous vacuoles in the living cell, possibly containing highly dispersed materials such as proteins and lipoids, as found by Simpson (1941) after the freezing-drying technique. Both the work of Simpson and our own would indicate far less concentration of proteins in the Golgi zone than in the surrounding cytoplasm. During the process of fixation there may be a distortion of the zone as Parat thought, and more recently, Worley; and the distorted "apparatus" may be fixed by any fixative that coagulates the cytoplasm around it. The similar appearance of the apparatus as shown by ultraviolet photography, whether the fixative be formalin or Da Fano, shows that the reduction of the silver on or in the apparatus subsequent to fixation depends, in all probability, not on the fidelity of the whole cell's fixation but on the character of the fixative used.

In conclusion, these studies of cells by means of ultraviolet photography give certain negative information as to the material in the Golgi zone: it does not appear that it is nucleoprotein or nucleic acid, except possibly in greater dilution than in the rest of the cytoplasm. They also suggest that successful silver impregnation after one fixative and not after another may be due, not to less faithful fixation of the cell, but to a more direct relation between the fixative and the silver. Finally it must be remembered that these studies were made exclusively on fixed material and there is much evidence that the morphology of the cellular constituents in such material does not coincide with that in living cells.

LITERATURE CITED

Hibbard, Hope, 1942. The "Golgi apparatus" during development in the stomach of Gallus domesticus. *Jour. Morph.,* **70**: 121–150.

Hibbard, Hope, 1945. Current status of our knowledge of the Golgi apparatus in the animal cell. *Quart. Rev. Biol.,* **20**: 1–19.

Lavin, George I., 1943. Simplified ultraviolet microscopy. *Rev. Sci. Instr.,* **14**: 375–376.

Monné, Ludwik, 1939a. Polarizationsoptische Untersuchungen uber den Golgi-Apparat und die Mitochondrien männlicher Geschlechtzellen einiger Pulmonaten-Arten. *Protoplasma,* **32**: 184–192.

Monné, Ludwik, 1939b. Über die Farbenveränderung der Mitochondrien und des Golgi-Apparates im Dunkelfeld. *Arch. exp. Zellforsch.,* **23**: 157–168.

Parat, Maurice, 1928. Contribution à l'étude morphologique et physiologique du cytoplasme. *Arch. d'Anat. Microsc.,* **24**: 74–357.

Simpson, William L., 1941. The application of the Altmann method to the study of the Golgi apparatus. *Anat. Rec.,* **80**: 329–343.

Worley, Leonard G., 1943a. The structure and function of the Golgi system in the living cells of developing molluscs. *Proc. Nat. Acad. Sci. Wash.,* **29**: 225–228.

Worley, Leonard G., 1943b. The relation between the Golgi apparatus and "droplets" in cells stainable vitally with methylene blue. *Proc. Nat. Acad. Sci. Wash.,* **29**: 228–231.

Worley, Leonard G., 1944a. Studies of the vitally stained Golgi apparatus. II. Yolk formation and pigment concentration in the mussel Mytilus californianus Conrad. *Jour. Morph.,* **75**: 77–101.

Worley, Leonard G., 1944b. Studies of the vitally stained Golgi apparatus. III. The methylene blue technique and some of its implications. *Jour. Morph.,* **75**: 261–289.

Worley, Leonard G., and E. K. Worley, 1943. Studies of the supra-vitally stained Golgi apparatus. I. Its cycle in the tectibranch mollusc Navanax inermis (Cooper). *Jour. Morph.,* **73**: 365–399.

DILUTION MEDIUM AND SURVIVAL OF THE SPERMATOZOA OF ARBACIA PUNCTULATA.* I. EFFECT OF THE MEDIUM ON FERTILIZING POWER

TERU HAYASHI

Department of Zoology, University of Missouri, Columbia, Missouri

INTRODUCTION

The investigations in sperm physiology may be roughly divided into two principal aspects. First, there is the problem of the role of the sperm cell in fertilization. Second, there is the problem of the survival of spermatozoa as a fundamental condition for the survival of the species. These two aspects each have their own long lists of investigations.

The study of the sperm cell in fertilization has produced one outstanding theory. This is the Fertilizin Theory of Lillie (1914). The course of investigations, past and recent, shows that this theory, although not completely confirmed and probably in need of modification, has been found useful by many workers in the field. According to the theory, the male germ cell is the carrier of a substance, the "sperm receptor," which is functional in the fertilization process. This substance is thought to combine with "fertilizin," an egg secretion. The complex of sperm-receptor-fertilizin then reacts with an "egg receptor" to form a three-way complex in the egg. The formation of this ternary complex initiates the fertilization reactions of the egg.

The study of sperm senescence, in contrast to the above, has yielded results which are, at best, unsatisfactory. Gray (1928a and b), who investigated the changes in metabolism of sperm under various conditions, reported that sperm in highly concentrated condition have a very low rate of respiration. If diluted, the sperm show a burst of metabolic activity. The greater the dilution, the more intense is this burst of action, although of shorter duration. Gray advanced the hypothesis that a large part of the sperm cell's internal supply of fuel was used up in the first burst of energy, so that the greater its intensity, proportionately shorter became the life of the spermatozoön. The initial burst of activity was in turn determined by the available "free space" in which the sperm cell could move, that is, by the dilution. In the limited space available to each cell in the concentrated suspensions, the sperm cell was only incompletely activated, and, hence, its life was prolonged.

This explanation cannot be applied without certain limitations. If it were, a single spermatozoön placed in an infinitely large volume of diluent would end its metabolism instantly. Further, "mechanical crowding" as an explanation is applicable only to the translatory or vibratory activity of the sperm and not to the respiratory activity. With all the known variables, such as oxygen and carbon dioxide tensions, rigidly controlled, Gray's evidence shows that when "free space" is available the rate of sperm respiration increases. "Mechanical crowding" is thus not an explanation for the changes in respiratory rate but a description of the conditions under which the respiratory rate is low. That is, it is logical to state that sperm are quiescent because they are forced to be immobile, but it is not logical to state that sperm respire at a high rate because they are no longer forced to be immobile. Such a statement has implications of teleology. There must exist an unknown factor which, under conditions of dilution, brings about the increased respiration of sperm. Undiluted sperm, therefore, must be a system composed of the cells plus the unknown factor. Dilution of the system, not the dilution of cells alone, brings about the respiratory activity of the spermatozoa.

---

* Work done as part of the requirement for the degree of Doctor of Philosophy.

The foregoing review shows that Lillie's fertilization studies have indicated the existence of a substance that determines the fertilizing power of the sperm cell. The review shows, too, that Gray's work has neglected one variable, the sperm cell medium, or a factor in that medium which affects the duration of metabolic activity of the sperm cell. It is the purpose of this research to present evidence for the existence of a single factor that influences the conservation of fertilizing power by sperm and the respiratory activity of sperm. The work is presented in two sections, the first section dealing with the fertilizing capacity of sperm, and the second with the respiratory activity of sperm.

The author is greatly indebted to Dr. Daniel Mazia for his guidance and helpful suggestions.

Materials and Methods

The materials used in the series of experiments to be described were the germ cells of the Atlantic sea-urchin, *Arbacia punctulata*. The general methods and precautions outlined by Just (1939) were followed carefully. To obtain the germ cells, the urchins were thoroughly washed in running sea water and running tap-water, after which they were dried carefully with clean cheese-cloth. A cut around the oral region disclosed the sex of the animal. If male, the sperm exuding from the genital pores were received in a dry stender dish; if female, the animal was allowed to shed the eggs into a stender dish filled with sea water.

The sperm suspensions for the earlier experiments were made according to the "drop" method of Lillie (1913). For greater precision in later experiments, sperm were "packed" by centrifugation at 3500 r.p.m. for 30 minutes. These packed sperm cells were drawn into a calibrated capillary tube. The tip of the capillary was wiped clean, and the contents were used to make the sperm suspension. The capillary was calibrated by taking up the same volume of re-distilled mercury and weighing the mercury accurately.

As a check on the constancy of this method, sperm counts were made. A unit quantity of packed sperm was suspended in one cc. of sea water, shaken thoroughly, and 0.01 cc. of Bouin's fixative added. This suspension was diluted one hundred times, and the number of sperm present counted in a haemocytometer chamber. The results are given in Table I, and it was found that the greatest deviation from the average was in the order of 6 per cent, a constancy not attainable by the "drop" method.

The seminal fluid used in the experiments was collected simply by drawing off the supernatant fluid from the packed "dry" sperm after centrifugation.

The egg suspensions were made by washing the eggs several times in sea water and allowing them to settle in the dish by force of gravity. Equal samples of the

TABLE I

Sperm count, using 0.00155 cc. packed sperm per cc. of sea water, diluted 100 times

Suspension	Number squares counted	Total counted	Average number per sq.	Conc. of packed sperm per cc.
No. 1	32	386	12.0	3.06×10^{12}
No. 2	32	346	10.8	2.76×10^{12}
No. 3	32	379	11.8	3.02×10^{12}
No. 4	32	360	11.2	2.86×10^{12}
No. 5	32	390	12.1	3.09×10^{12}
No. 6	32	359	11.2	2.86×10^{12}
Average				2.94×10^{12}

settled eggs were diluted in varying amounts of sea water, mixed to give homogeneity, and aliquots were removed with a calibrated pipette. From the number of eggs present per unit length, the total number of eggs could be calculated. One drop of a suspension of suitable egg-concentration was placed in 5 cc. of sea water. Generally, the number of eggs in one insemination test was 750–1000. As Lillie (1915a) had shown, variations of this order in the total number of eggs used in the inseminations do not affect the final results appreciably. Since a fresh egg suspension was used for the insemination tests at any one time, the tests at two different times used different suspensions whose concentrations varied somewhat, so that the results were possibly not comparable. Those tests run at any one time used the same egg suspension, and, therefore, the results were comparable to each other.

For the insemination, a unit quantity of the sperm suspension in a pipette was carefully squeezed out over the eggs, and the whole dish gently and uniformly stirred. For determination of fertilizing power, the percentage of eggs activated was calculated by counting a minimum of 200 eggs.

Widely diverging types of experiments were made in the course of this investigation, each type entailing its own methods and techniques. Because of this, other methods and techniques will be described in connection with particular experiments. Each typical experiment to be presented in the following section was one of a minimum of five experiments all giving similar results.

EXPERIMENTS AND RESULTS

The seminal fluid factor and the survival of sperm

Past researches have shown that sperm in the undiluted condition freshly-exuded from the testes are immobile, and that the sperm manifest intense activity upon dilution with sea water. Subsequently, the fertilizing power of the sperm cells declines sharply and within a relatively short time. Workers in the past had diluted sperm fresh from the testes with sea water. Since the medium seemed to be a variable in this type of dilution, and since sperm cells in the testes were suspended in a liquid medium, a factor influencing the fertilizing capacity of sperm cells was sought in the seminal fluid.

To examine the effect of the seminal fluid on the fertilizing power of sperm, a series of experiments was done using sperm suspensions of the same concentration in seminal fluid and in sea water. These suspensions were then tested at different time intervals for their fertilizing power. In the experiment shown in Table II, a 0.4 per cent sperm suspension (according to the terminology of Lillie) was used. One drop of the suspension was used to inseminate 750–1000 eggs. The formation of the fertilization membrane was used as the index of activation of the egg.

The effect of the seminal fluid in promoting the survival of the sperm was apparent even after five hours, and after 12 hours, when the sperm in sea water were completely non-functional, a large number of those in the seminal fluid were still capable of bringing about activation. At each test, microscopic observation revealed that the per cent activation of eggs was approximately directly proportional to the number of motile sperm.

TABLE II

Activation of eggs by sperm suspensions of 0.4 per cent concentration in seminal fluid and sea water

Medium	Per cent activation					
	10 a.m.	11 a.m.	3 p.m.	5 p.m.	8 p.m.	10 p.m.
Sea water	100	100	46	22	0–2	0
Sem. fluid	100	100	99	100	99	95

The maintenance of fertilizing power of the sperm cells was a function specific for the seminal fluid. Experiments were made using the perivisceral fluid as the suspension medium. The perivisceral fluid was found to have a toxic effect on the retention of fertilizing power by sperm.

It seemed clear that in the seminal fluid an unknown factor was enabling the sperm to retain their fertilizing power for a long period of time. In view of the work of Cohn (1918), a check of the effect of pH became necessary. The pH of the seminal fluid was measured electrometrically with McGinnis' electrode. A number of such measurements showed the pH of seminal fluid to vary between 7.6 and 7.9.[1] Experiments were done comparing the survival of sperm in seminal fluid and sea water acidified to the same pH as the seminal fluid sample.

In the same experiments, another chemical property of the seminal fluid was investigated, namely, the heat-sensitivity. A sample of the seminal fluid in a test tube was heated at 100° C. for ten minutes, the seminal fluid allowed to cool to room temperature, and this heated seminal fluid was tested for its effect on the survival of sperm.

The results of experiments are summarized in Table III. The dilution used was one drop of centrifuged sperm to 5 cc. of medium. The pH of this seminal fluid sample was 7.72; the sea water (pH 8.0) was acidified to 7.7 by the addition of 11 drops of 0.1 N HCl to 100 cc. of sea water. All the suspensions were made at 5 p.m.

The results showed that acid sea water maintained the fertilizing power of the sperm only slightly longer than normal sea water and not nearly so long as the

---

[1] Done by Mr. M. E. Smith, of the MBL staff.

TABLE III

The effects of pH, heated seminal fluid on the survival of sperm, as shown by time measurements of the fertilizing power

Medium	Per cent activation				
	5 p.m.	9 p.m.	10:30 p.m.	4 p.m.	10 p.m.
Sea water	100	100	14	0	0
Sem. fluid	100	100	100	97	73
Heated fluid	100	0	0	0	0
Acid s.w.	100	100	40	2	0

seminal fluid. The heated seminal fluid, on the other hand, had clearly lost the function of promoting the survival of the sperm cells. It was evident that pH was not the effective factor in the seminal fluid and that the effective factor was heat-sensitive.

This heat sensitivity led to the suspicion that the unknown factor was protein. To test this hypothesis, the seminal fluid was saturated with ammonium sulfate. A faintly rose-colored precipitate resulted from this treatment. This precipitate was filtered off, and the residue on the filter paper dissolved in a volume of sea water equal to the original volume of seminal fluid. The sea water containing the residue was then dialyzed against fresh changes of sea water in the refrigerator for 30 hours. The dialyzing membrane was commercial sausage skin (Cenco). This treatment removed the ammonium sulfate. The liquid inside the dialysis bag, essentially an artificial seminal fluid, was then used as the suspending medium for the sperm.

As controls for this experiment, various other media were used to suspend equal concentrations of the same sperm sample. For the first of these, the filtrate of the seminal fluid (seminal fluid minus the precipitated material) was also dialyzed against sea water for the same length of time as the residue solution, and this "dialyzed filtrate" was used as a suspending medium for the sperm. Normal sea water, acid sea water, and natural seminal fluid were also run as controls. The dilution used was one drop of centrifuged sperm to 10 cc. of medium, and the pH was carefully checked in each case.

The results (Table IV) showed that spermatozoa in the "artificial seminal fluid" retained their fertilizing power nine hours longer than did the sperm in sea water. From the data, it was concluded that the seminal fluid factor was precipitable with ammonium sulfate and non-dialyzable. The earlier conclusion as to the negligible effect of pH was confirmed in this experiment.

The idea of the seminal fluid factor's being protein seemed to be borne out and warranted an analysis of the seminal fluid for its protein content, along with determinations of other physical and chemical properties. For determination of protein, Folin's micro-Kjeldahl with direct Nesslerization was used, the solutions being compared in a photoelectric colorimeter. The results showed 2.5 mg. protein per cc. of 100 per cent seminal fluid. The pH of the seminal fluid was found to vary between 7.6 and 7.9 as compared to the pH of sea water, which varied from 7.9 to 8.1.[2] The freezing point of seminal fluid was $-1.715°$ C. as compared to that of

---

[2] Done by Mr. M. E. Smith, of the MBL staff.

TABLE IV

The effects of various media on the survival of sperm, as shown by insemination tests

Medium	pH	Per cent activation					
		0.5 hrs.	5.5 hrs.	10.0 hrs.	15.5 hrs.	24.0 hrs.	26.0 hrs.
Sea water	8.0	98	92	49	13	0	0
Acid sea water	7.7	98	87	72	68	0	0
Sem. fluid	7.6	100	97	100	100	98	35
Dial. residue	7.8	99	96	98	85	19	4
Dial. filtrate	7.8	93	80	20	5	0	0
Eggs tested		98	98	100	100	98	100

sea water, which was −1.892° C.[3] Chloride analysis showed the sea water to contain 0.508 moles per liter, while the seminal fluid contained 0.590 moles per liter.[4] Analysis for glucose (reducing sugar) showed the seminal fluid to contain less than 10 gamma in 5 cc.

These results suggested as one possibility that the action of seminal fluid on the sperm could be attributed to the osmotic pressure difference between the seminal fluid and the sea water. The demonstrated heat-sensitivity of the seminal fluid factor, however, ruled this possibility as unlikely, as did the prolonged dialysis of the last experiment given, for such treatment would equalize the osmotic pressure of the seminal fluid with that of the sea water.

The difference in chloride content between the seminal fluid and sea water was not considered as a factor in prolonging the fertilizing power of the sperm cells. The prolonged dialysis described earlier would have equalized the chloride concentration of the sea water and the "artificial seminal fluid" of Table IV, yet these two media had markedly different effects upon the sperm cells. Also, the demonstrated heat sensitivity of the seminal fluid factor indicated that it was not chloride.

The effective seminal fluid factor therefore seemed to be protein, but protein, by its presence, would establish a colloidal osmotic pressure which might be the agency acting on the sperm.

In Table IV, it may be noted that the "dialyzed residue" was not as effective as the natural seminal fluid. There are several possible explanations. First, during the prolonged dialysis, some of the protein may have been denatured, a point to be checked in future investigations. Second, the concentration of the factor in the "artificial seminal fluid" was probably not equal to that in the natural medium, due to some loss of protein in handling, and difficulties in volume control in dialysis.

At this point, attention should be called to the fact that still another possibility existed as to the manner in which the seminal fluid functions. This was the question of nutrition of the sperm by the seminal fluid. This question will, however, be taken up in the discussion.

There remained one mode of action of the seminal fluid factor hitherto uninvestigated. The results of the experiments already described validated the assumption that the seminal fluid factor acted in some manner upon the surface of the sperm cells.

---

[3] Done by Dr. Jay A. Smith, of the MBL staff.

[4] Done by Mr. J. Weissiger, of the MBL staff.

Observations made during attempts to measure sperm activity in a capillary tube showed spermatozoa to be positively thigmotropic to glass surfaces. At the instant of contact, the spermatozoön lost a large part of its activity and rotated slowly about its point of contact. The observation seemed to show the presence of a surface active substance on the head of the spermatozoön. This fact, previously observed by Buller (1902), led to the following experiment.

Three suspensions of sperm of equal concentration were made in sea water. Suspension No. 1 was left untreated. Glass powder was added to suspensions No. 2 and No. 3. All three suspensions were shaken simultaneously and placed in the refrigerator, where the powdered glass was allowed to settle for three hours. Insemination tests were run to determine the relative sperm populations in these three suspensions. Qualitative microscopic observations on sperm population were also made at each dilution of the original suspensions as a check.

As shown in Table V, the results indicated that the sperm population in the second and third suspensions was greatly reduced, a result confirmed by microscopic observation. It was possible that the glass powder injured a large part of the total sperm population, but the absence of significant numbers of injured sperm seemed to indicate that the glass powder removed the missing sperm by adhesion.

TABLE V

Activation of eggs by progressive dilutions of sperm suspensions treated with glass powder as compared to untreated sperm suspension

Suspension	Undiluted	1:1 Dilution	3:1 Dilution
No. 1	100	100	100
No. 2	96	61	27
No. 3	100	75	35

A similar experiment was made to test the surface activity of seminal fluid protein, since the proposed surface-action implied the identity of sperm-surface-substance and seminal fluid protein. A sample of seminal fluid was divided into three portions. Portion No. 1 was left as the untreated control. Glass powder was added to portion No. 2, the portion shaken thoroughly, and the glass powder filtered off with Whatman No. 5 filter paper. Portion No. 3 was shaken three times, each time with fresh glass powder and filtered free of glass each time. These seminal fluid portions were then used to make sperm suspensions of equal concentration and tested for the maintenance of the fertilizing power. The results are given in Table VI.

Clearly, the glass powder removed the sperm-longevity factor from the seminal fluid, so that seminal fluid protein, too, seemed to be surface-active on glass. Although the experiments of Tables V and VI did not completely establish the identity of the seminal fluid factor and the substance on the surface of the sperm, they did show that both substances were apparently surface-active.

In furtherance of this line of thought, experiments were made to learn whether sperm in sea water gave off their surface substance into the surrounding medium.

TABLE VI

Removal of the factor from seminal fluid with glass powder

Medium	Per cent activation						
	0.0 hrs.	4.0 hrs.	9.5 hrs.	12.0 hrs.	14.5 hrs.	24.0 hrs.	28.0 hrs.
Sea water	100	100	78	65	40	0	0
Portion No. 1	100	100	100	100	100	100	95
Portion No. 2	100	100	100	80	54	5	0
Portion No. 3	100	99	1	0	0	0	0
Eggs tested	100	100	100	100	100	100	100

In one type of experiment, a heavy suspension of sperm in sea water was allowed to stand for several hours. The sperm were then removed by centrifugation and the supernatant fluid tested as a sperm medium. In another type of experiment, the above procedure was repeated several times, the supernatant fluid used to support a fresh sample of sperm after each centrifugation. After the final centrifugation, the supernatant fluid was tested for its effect on fresh sperm. In all cases, the results were negative. Such "sperm washings" had neither a detrimental nor favorable effect on the maintenance of the fertilizing power of the sperm.

There remained one other point of investigation in the survival time of spermatozoa. Observations had shown that seminal fluid protein, even in low concentration, was effective in maintaining spermatozoa. Gray (1928a) had postulated a "mechanical crowding" effect as the primary factor in the survival of sperm. Since he used "dry" sperm, which was composed of about 60 per cent seminal fluid, there arose the possibility that the longer survival of the more concentrated sperm had as its cause, not "mechanical crowding," but the larger amounts of seminal fluid protein carried over in the "dry" sperm. A test of this possibility followed.

A sperm suspension in seminal fluid was made by suspending 0.025 cc. of packed sperm in one cc. of seminal fluid. A second suspension was made by taking 0.2 cc. of the first suspension and adding it to another one cc. sample of seminal fluid. This serial dilution was repeated twice more, to make four sperm suspensions, all in seminal fluid. The operation was carried out quickly, the last suspension made within a minute of the first. The final concentrations of the four suspensions were, in Lillie's terminology, approximately 5 per cent, 1 per cent, 0.2 per cent, and 0.04 per cent, since packed sperm contained approximately twice the amount of sperm per unit volume as did the "dry" sperm used by Lillie. The insemination tests were made at the same dilution, each of the more concentrated suspensions being diluted to the lowest concentration of 0.04 per cent. One drop of this final suspension was used to inseminate the eggs. The results are given in Table VII.

A study of these results as compared to those of Gray showed that, even though Gray's results might be partly explained as the action of seminal fluid protein, "mechanical crowding" did seem to play a part in determining the life-span of the spermatozoa. However, it may be pointed out that this "crowding effect" seems to be non-linear in relation to the concentration, and is most apparent at extreme dilutions.

TABLE VII

The effect of concentration on the survival of sperm in seminal fluid

Suspension concentration	Per cent activation					
	0.5 hrs.	4.0 hrs.	7.5 hrs.	18.0 hrs.	23.0 hrs.	30.0 hrs.
5 per cent	88	90	78	77	85	91
1 per cent	81	85	83	79	83	72
0.2 per cent	90	92	84	80	49	13
0.04 per cent	92	95	86	45	10	0

The seminal fluid factor and its role in fertilization

In the course of the preceding experiments, sea water suspensions of sperm used to test the eggs showed a contrasting behavior as to fertilizing power. The individual spermatozoön in seminal fluid appeared to have a greater fertilizing power than the spermatozoön in sea water. An experiment was devised to investigate this more closely.

A volume of 0.025 cc. of packed sperm was suspended in one cc. of seminal fluid. Immediately after the suspension was made, one drop of the suspension was used to inseminate approximately 1000 eggs. Serial dilutions were made as for the previous experiment, but as each new suspension was made, one drop was used to inseminate approximately 1000 eggs. A sea water control was run, dilution and inseminations being made in the same way (Table VIII).

TABLE VIII

A comparison of the fertilizing power of sperm in seminal fluid and sperm in sea water

Medium	Per cent activation			
	1st dilution	2nd dilution	3rd dilution	4th dilution
Sea water	100	97	37	12
Sem. fluid	100	99	100	81

The results proved that there was a strong difference in the fertilizing power of the sperm cells in seminal fluid as compared to those cells in sea water. This difference became even more pronounced when the original concentrated suspensions were allowed to stand for ten hours, as shown in Table IX. Only the most concentrated suspensions in the seminal fluid and the sea water were kept. The dilutions were made anew.

The interpretations of these results were rather complex and will be discussed in a later section.[5]

The apparent increased fertilizing power of the sperm in the seminal fluid indicated that seminal fluid factor might be directly concerned with the fertilization process. It was recalled that Lillie (1915) had given as one of the criteria for the "sperm receptor" the power to "bind" agglutinin from the egg. Lillie meant by this

---

[5] See page 175.

TABLE IX

A comparison of the fertilizing power of sperm in seminal fluid and sea water after 10 hours

Medium	Per cent activation			
	1st dilution	2nd dilution	3rd dilution	4th dilution
Sea water	100	86	7	0
Sem. fluid	100	100	98	79

that if the agglutinin were treated with the "sperm receptor" solution (here the seminal fluid, presumably), the action of the agglutinin on the sperm would be greatly reduced. This experiment was done, with the expectation that, if the seminal fluid factor and the "sperm receptor" were one and the same, the agglutinating action of the egg secretion would be reduced.

A series of dry watch glasses was arranged. In the first, two drops of seminal fluid and two drops of egg-water were thoroughly mixed. Two drops of this mixture were then removed to the next watch glass and diluted with two drops of sea water. This treatment was repeated down the series. For the control, sea water was used instead of seminal fluid. For the test, a drop of a standard sperm suspension (0.00155 cc. packed sperm per cc. of sea water) was placed in the watch glass, out of contact with the mixture. The watch glass was then placed under the objective of the microscope, the two liquids (sperm suspension and sea water-seminal fluid mixture) shaken together, and the reaction of the sperm noted. In the following table, + indicates a positive agglutination, − a negative agglutination, and ± uncertain. The number of + symbols indicates the intensity of the reaction.

TABLE X

The agglutination reaction induced by dilutions of egg-water-seminal fluid mixtures, as compared to those induced by egg-water-sea water mixture of the same dilutions

Dilution	Sea water egg-water	Sem. fl. egg-water
1	+++	++++
1/2	+++	++++
1/4	+++	+++
1/8	++	+++
1/16	+	++
1/32	±	+
1/64	−	+
1/128	−	±

Instead of having its action on the sperm reduced, the results revealed that egg-water treated with seminal fluid had, if anything, a more powerful agglutinating power than the sea water-treated egg-water. In any event, the agglutinating power was not reduced. The only conclusion possible from these results seemed to be that the seminal fluid factor was not the "sperm receptor" of Lillie.

However, the data given indicated that the sperm reaction in the seminal fluid-egg-water mixture was more intense than the corresponding reaction of sperm in the egg-water sea water mixture. This phenomenon was put to a quantitative test.

Standard suspensions of sperm were made in seminal fluid and in sea water. The concentration was 0.00155 cc. packed sperm per cc. of medium. These suspensions were allowed to stand at room temperature (25° C.). At intervals, a drop from either one or the other of the suspensions was placed on a watch glass, out of contact of a mixture of one drop of egg-water and two drops of sea water. The liquids were shaken together under the microscope, and the time of agglutination (from onset to reversal) was taken with a stop-watch. The results are summarized in Table XI.

TABLE XI

The agglutination time of sperm suspended in seminal fluid as compared to that of sperm suspended in sea water

Time Tested	Agglutination time in seconds	
	Sperm in sea water	Sperm in seminal fluid
p.m.		
3:00	90	120
3:06	63	69
3:10	61	115
3:18	82	98
3:40	49	111
3:44	53	95
3:50	47	91
3:57	63	90
4:05	61	97
4:40	91	86
4:50	86	75
5:15	34	120
5:30	71	115
7:30	75	76

The data show that, on the average, the sperm in the seminal fluid remained agglutinated for a longer time than the sperm in sea water. Although the results showed wide variation, the contrast between the two sperm suspensions was quite striking. From the results, it seemed reasonable to conclude that seminal fluid had changed the sperm surface in such a way as to bring about a stronger reaction with agglutinin.

DISCUSSION

The seminal fluid factor and sperm motility

Gray (1928a) observed that the motility of the sperm of *Echinus miliaris* was in no way impaired when suspended in seminal fluid, and he stated conclusively that the seminal fluid possessed no chemical or physical properties inhibiting sperm motility. He prepared the seminal fluid, which he called "testicular plasma," by strong centrifugation of the "dry sperm," the same method employed in this investigation.

The experiments and observations of the present study confirm Gray. The earlier results of the work, given in a preliminary note (Hayashi, 1940), showed that the sperm of *Arbacia punctulata* were motile in seminal fluid, with an intensity of movement at least equal to that exhibited by sperm in sea water. Moreover, this motility persisted for a longer time in the former medium. That sperm are active in seminal fluid was confirmed by respiration studies (results to be given in a subsequent report), for it was found that the respiratory activity of sperm was maintained at a higher level for a longer time in seminal fluid than in sea water. Therefore, it may be stated conclusively that sperm cells of *A. punctulata* and *E. miliaris* are fully active in seminal fluid.

The observations and conclusions of Southwick (1939a) were found to be in conflict with these results. This worker found that sperm of *Echinometra subangularis* were immobile when suspended in the seminal fluid of the same species. He concluded that there was present in the seminal fluid a substance which inhibited the activity of the sperm.

Hartman (1940) and Hartmann, Schartau, and Wallenfels (1940) confirmed Southwick on the presence of the inhibiting factor not only in the seminal fluid, but also in the sea water that had contained large numbers of spermatozoa. Their work, however, was done with the sperm of *Arbacia pustulosa*. In addition to confirming Southwick, Hartmann et al. stated that the function of the inhibiting factor was to neutralize echinochrome A, a sperm-activating substance from the egg.

For several reasons, the conclusions of these workers do not seem to be justified. In the first instance, Southwick's own observations reveal that freshly-exuded "dry sperm" possess an intense vibratory activity, an apparent contradiction to his own conclusion. This activity is lost after a few minutes. A number of investigators have published observations pertinent to these phenomena. Thus, Harvey (1930) showed that sperm of *Arbacia punctulata* in oxygen-free sea water were immobile; when oxygen was introduced the sperm regained their motility. Lillie (1913) demonstrated that sperm of Nereis and Arbacia lost their motility in the presence of carbon dioxide. Dungay (1913), using Nereis and Arbacia, Fuchs (1914) with *Ciona intestinalis,* and Cohn (1918) with *A. punctulata* proved that acid media had a deleterious effect on sperm. Finally, Carter (1931) working with *Echinus esculentus* and *Echinus miliaris,* and Taylor and O'Melveny (1941) with *Strongylocentrotus purpuratus* and *Lytechinus anamesus* obtained experimental proof that acid conditions lowered the respiratory activity of sperm.

In view of the results of these investigators, the brief activity of the sperm noted by Southwick seems to be attributable to the newly-made contact of the sperm with oxygen upon shedding. The subsequent inactivation of the sperm has its probable explanation in the acid conditions induced by the carbon dioxide production of the sperm.

Furthermore, the papers of Southwick and the Hartmann school yield no figures on the pH of the media used by these workers, nor do their texts give any evidence that this factor had been controlled. In addition, the conclusions of Hartmann et al. concerning the effect of echinochrome have not been confirmed by the experiments of Tyler (1939b) and Cornman (1940, 1941). The former worker found that neither echinochrome nor spinochrome would stimulate the respiration of sperm of *S. purpuratus*. The latter showed that crystalline echinochcrome did not increase the motility of the sperm of *A. punctulata*. The paradoxical results of Tyler and Cornman as opposed to Hartmann et al. may be attributed to species difference. However, it is clearly possible that echinochrome does not activate sperm. The non-existence of a sperm-activating function by echinochrome seems to weaken the argument for the existence of a substance neutralizing that activating factor.

Because of these considerations, the concept of a sperm-inhibitor in the seminal fluid seems to be questionable. In the light of parallel experimental results as regards sperm motility and respiratory activity (Hayashi, unpublished), it is concluded that there is no inhibitor of sperm motility in the seminal fluid of *A. punctulata.* This conclusion does not deny the inhibiting effects of hydrogen ions, the influence of which on the increase of the life-span of the sperm has been shown to be insignificant. To restate the conclusion: excluding the hydrogen ion factor, there is no inhibitor of sperm motility in the seminal fluid of *A. punctulata.*

The seminal fluid factor in its relation to the activating capacity of the sperm

Various experiments have proved that spermatozoa suspended in seminal fluid retain their capacity to activate eggs longer than sperm cells suspended in sea water (Tables II, III, IV). The factor in the seminal fluid responsible for the effect is not found in the perivisceral fluid, the factor is not the pH of the medium, and the factor is heat-sensitive (Table III). The seminal fluid factor is also non-dialyzable and precipitable with ammonium sulfate (Table IV). On the basis of these results, it may be tentatively stated that the seminal fluid factor is protein. However, the usual protein tests have not been made, so that this conclusion cannot be drawn with any finality, even though the conclusion is strongly supported by positive micro-Kjeldahl analyses indicating protein content in the order of 2.5 mg. protein per cc. of seminal fluid.

The seminal fluid factor, if protein, may act on the sperm cells in several ways. The factor may serve as a source of nutrient for the sperm, it may act on the sperm through the agency of the colloidal osmotic pressure which its presence establishes in the seminal fluid, or it may act through adsorption on the sperm surface. It is necessary to consider these possibilities carefully, if the mechanism of the action of the seminal fluid factor is to be clarified.

The possibility of the seminal fluid factor's acting as a nutrient will be taken up more fully in a later publication on the effect of the seminal fluid on the respiration of sperm. The statement can be made here that these studies indicate that the factor does not act as a nutrient for the sperm. Likewise, the probable protein nature of the factor argues against the idea of nutrition, for the large size of the molecule would prevent its absorption by the sperm. The fact that seminal fluid contains no reducing sugar is further support for the belief that the seminal fluid affords no nutritive elements for the sperm cells.

The question of the effect of colloidal osmotic pressure in prolonging the functional life of the sperm cell is unsettled. Although the further experimental results on the surface activity of seminal fluid substance validate the conclusions drawn, it is admitted that the effects of colloidal osmotic pressure on sperm longevity is still an open question.

The experimental results given in Tables V and VI constitute support for the idea of surface-action of the seminal fluid factor. The data show that both the sperm surface and the seminal fluid factor are surface-active on glass, and they indicate the possible identity of the sperm-surface-substance and the seminal fluid factor.

The foregoing considerations point strongly to the conclusions that the seminal fluid factor is protein and that it is present both in the seminal fluid and on the sperm surface. Since the seminal fluid factor enables the sperm to retain their fertilizing function, it seems logical to infer that the seminal fluid protein plays a part directly in the fertilization process. The data of Tables VIII and IX give support to this idea. The experiment of Table VIII reveals the fact that, with the same amounts of sperm, a higher percentage of activation of eggs is achieved by sperm in seminal fluid than by sperm in sea water. Since the experiment was so arranged that the insemination tests were made immediately after the mixing of each solution, the possibility that a large number of sperm in the sea water were immobilized seems unlikely. The conclusion most compatible with the results is that the individual spermatozoön in seminal fluid possesses a greater fertilizing capacity than his fellow in sea water. The mere act of dilution in sea water, therefore, seems to have removed a large part of the activating substance from the surfaces of the sperm cells, reducing their individual activating power.

The idea of variation of the activating power of the individual sperm cell was first expressed by Glaser (1915). He diluted sperm serially in sea water, and found that several sperm cells were required to activate one egg cell, even though only one spermatozoön was required to bring about the biparental effect. Lillie (1915) found that when he used the same method of dilution as did Glaser, the fertilizing power of the suspension was far less than an equal concentration of sperm in a suspension diluted in one step. Although these two workers disagreed in their conclusions, their results point to the validity of Glaser's interpretation, which is confirmed in the present study.

Table IX shows the relative fertilizing powers of suspensions in sea water and seminal fluid after they had been aged for 10 hours. If the results are compared to those of Table VIII, it will be seen that the fertilizing capacity of the seminal fluid sperm suspension was not affected by the aging period but that the fertilizing capacity of the sea water suspension was markedly reduced. There are two possible explanations for the enhanced difference in the activating power of the two suspensions, both of which probably contribute to the effect. It is possible that in the 10-hour aging period, large numbers of the sperm cells in the sea water suspension were immobilized, so that they could not penetrate the jelly envelope surrounding each egg. Thus, the number of sperm cells making actual contact with the egg surface was reduced. The final result would be a decreased percentage of activation. The second possibility is the conclusion derived from the analysis of Table VIII, namely, that a substance functional in activation was removed from the sperm surface. During the 10-hour period, this removal presumably continued, so that the activating power of the individual sperm cell was further reduced. Therefore, even if all the spermatozoa remained motile and capable of making contact with the egg, more sperm cells per egg would be required for activation, and the end results would be a decreased percentage of activation. The experiment, therefore, tends to support the idea of an egg-activating substance on the sperm surface, and, also, shows the close relationship between the motile activity and the activating power of the sperm cell.

Many investigators have postulated the existence of a substance on the surface of the sperm and considered that it was protein in nature. Buller (1902), from observations of the sperm of various echinids, reported that the sperm surface was surface-active, not only on glass, but also on air bubbles. Lillie (1913) discovered that in the presence of egg secretions, the male germ cells of Arbacia and Nereis became agglutinated. He concluded that the surface of the sperm cell was "sticky." The marked similarity of the agglutination phenomenon to an immunological reaction may be taken to be a strong indication for the protein nature of the responsible agent on the surface of the sperm cell.

More direct evidence came from the work of Popa (1927). Using histochemical technique, this worker concluded that the surfaces of Nereis and Arbacia sperm were covered with a layer of lipo-protein.

Mudd, Mudd, and Keltch (1929) investigated the surface charge of the sperm cells of various echinids. Using the cataphoresis chamber, they reported that the sperm surface was negatively charged. This negativity they found to be increased after agglutination with egg-water. They concluded that their method made possible the detection of substances on the sperm surface.

Henle (1938) and Henle, Henle, and Chambers (1938) found that heat-sensitive antigens existed on the surface of sperm heads. Their work was done with mammalian sperm. Tyler and O'Melveny (1941) obtained rabbit anti-serum by injection of whole sperm of *S. purpuratus* and *L. anamesus*. The anti-serum was found to agglutinate the sperm of these species. These immunological studies again pointed to the protein nature of the sperm-surface-substance.

The evidence cited is not, perhaps, a complete list. The investigations provide enough experimental data, however, to warrant the tentative conclusion that the sperm-surface-substance is protein in nature.

This sperm-surface-substance and the seminal fluid factor may possibly be identical (Tables V and VI). A strong indication of identity could be established if it were shown that the seminal fluid factor alone can activate eggs. Experiments have been started to investigate this possibility, but as yet no conclusive results have been obtained. Comparable work in this direction has not been done. The effect of protein extracts on egg surfaces was investigated by Favilli (1932) and by Hartmann, Schartau, and Wallenfels (1940), while Sampson (1926) reported the activation of eggs by dialysates and filtrates of sperm suspensions. Since her activating factor was dialyzable, and therefore non-protein, it cannot be compared to the seminal fluid factor. In addition, the remarks of Just (1922, 1928, 1929a and b) criticizing the auto-parthenogenesis of Glaser (1914) and Woodward (1921) are equally applicable to the work of Sampson.

Another possible method of establishing the identity of seminal fluid protein and the sperm-surface-substance is to obtain rabbit anti-serum by the injection of seminal fluid. If the anti-serum thus obtained had the power to agglutinate sperm, the results would constitute substantial evidence for the argument that seminal fluid protein and protein on the sperm surface were the same substance. The experiment, however, was not done.

The identity, therefore, is not established, although there is some evidence in this direction (Tables V and VI). Aside from this point, however, there are experimental results throwing light on the origin of and possible relation between the seminal fluid factor and the sperm surface substance. It would be interesting to know whether these substances are secreted by the sperm cells or not, and whether the sperm-surface substance establishes the seminal fluid substance by passing off into the seminal fluid, or whether the seminal fluid substance establishes the sperm-surface substance by adsorption on the sperm surface.

Numerous investigators have reported that sea-urchin sperm give off a substance into the surrounding sea water (Lillie, 1914 and 1915; Southwick, 1939; Hartmann, 1940; Hartmann, Schartau, and Wallenfels, 1940) and that this substance showed protein characteristics (Frank, 1939; Tyler and O'Melveny, 1941). All investigators agreed that the substance showed the properties of "antifertilizin" or the power to "bind" the agglutinin of egg-water so that the agglutinating effect on sperm was reduced.

The question here posed is: does this substance from the sperm cells have the properties of the seminal fluid substance? The point was tested by an experiment in which a sea water suspension of sperm was allowed to stand for several hours, the sperm cells removed by centrifugation, and a fresh sample of sperm suspended in the medium. The results were negative. This medium was not effective in prolonging the fertilizing capacity of sperm, and therefore did not have the properties of the seminal fluid substance.

There is the converse question: Does the seminal fluid substance have the antifertilizin property of the substance coming off the sperm cell? Again, the results were negative (Table X).

The substance coming off the sperm cell does not have the properties of the seminal fluid substance. The results of these experiments indicate, therefore, that a sperm substance does not establish the seminal fluid substance, so that the seminal fluid factor does not have its origin in the sperm cell. The same negative answer as to the origin of the sperm-surface substance cannot be given.

However, the fact that the substance coming off the sperm surface has different properties from the seminal fluid substance signifies nothing regarding the properties of the sperm substance while on the sperm surface. This substance on the surface of the sperm cell is surface-active on glass, as is the seminal fluid substance (Tables V and VI). The seminal fluid factor also enables the sperm cell to maintain its fertilizing capacity longer (Tables II, III, IV) and seems to enhance the fertilizing power of the individual spermatozoön. In addition, the seminal fluid factor affects the surface of the sperm so that the time of agglutination, or the reaction with agglutinin, is increased (Table XI).

These facts point to a tentative explanation of the relation between the seminal fluid factor and the sperm-surface substance. It is possible that a protein substance, originally present in the seminal fluid, is adsorbed on the surface of the sperm cell, thus influencing the fertilizing power of the sperm cell, as well as rendering the surface of the sperm cell surface-active. By this adsorption also, the sperm surface is rendered capable of greater reactivity with fertilizin. The subsequent loss of this substance from the sperm cell results in the loss of fertilizing power and the presence of antifertilizin in the sperm medium. The antifertilizin would be, according to this scheme, a substance changed in certain properties from the original seminal fluid substance.

Summary

1. A factor is present in the seminal fluid of *Arbacia punctulata* which prolongs the fertilizing capacity of the sperm cells of the same species.

2. The factor, which is not found in the coelomic (perivisceral) fluid, is heat-sensitive, precipitated by saturation with ammonium sulfate, non-dialyzable, and surface-active on glass. Since micro-Kjeldahl analysis of the seminal fluid gives positive results corresponding to 2.5 mg. protein per cc. of seminal fluid, it is tentatively suggested that the factor is protein.

3. Seminal fluid has a pH range of 7.6 to 7.9, its osmotic pressure is approximately 10 per cent lower than sea water, and its content of reducing sugar is negligible.

4. In equivalent concentration and immediately after suspension the fertilizing capacity of the individual spermatozoön is greater in seminal fluid than in sea water.

5. Seminal fluid does not contain antifertilizin since it does not neutralize the agglutinating action of egg-water; indeed, this action is intensified.

6. A tentative mechanism, based on the adsorption of a fertilizing substance and its removal from the surface of the sperm cell, is suggested to explain the experimental results. It is proposed that the seminal fluid factor is this fertilizing substance before adsorption and while on the surface of the sperm; it becomes changed upon removal from the sperm surface.

LITERATURE CITED

Buller, A. H. R., 1902. Is chemotaxis a factor in the fertilization of the eggs of animals? *Quart. Jour. Micr. Sci.*, **46**: 145–176.

Carter, G. S., 1931. Iodine compounds and fertilization. II. The oxygen consumption of suspensions of sperm of Echinus esculentus and Echinus miliaris. *Jour. Exp. Biol.*, **8**: 177–192.

Cohn, E. J., 1918. Studies in the physiology of spermatozoa. *Biol. Bull.*, **34**: 167–218.

Cornman, Ivor, 1940. Echinochrome as the sperm-activating agent in egg-water. *Biol. Bull.*, **79**: 365.

Cornman, Ivor, 1941. Sperm activation by Arbacia egg extracts with special reference to echinochrome. *Biol. Bull.*, **80**: 202–207.

Dungay, Neil, 1913. Effect of injury upon sperm. *Biol. Bull.*, **25**: 213–260.

Favilli, G., 1932. The influence of organ extracts on cell permeability. *Jour. Cell. Comp. Physiol.*, **2**: 1–10.

Frank, J. A., 1939. Some properties of sperm extracts and their relationship to the fertilization reaction in Arbacia punctulata. *Biol. Bull.*, **76**: 190–216.

Fuchs, H. M., 1914. The action of egg secretions on the fertilizing power of sperm. *Arch. f. Entw. der Org.*, **40**: 205–252.

Gee, Wilson, 1916. Effects of acute alcoholization on the germ cells of Fundulus heteroclitus. *Biol. Bull.*, **31**: 379–406.

Glaser, Otto, 1914. On auto-parthenogenesis in Arbacia and Asterias. *Biol. Bull.*, **26**: 387–409.

Glaser, Otto, 1915. Can a single spermatozoön initiate development in Arbacia? *Biol. Bull.*, **28**: 149–154.

Gray, J., 1928a. The effect of dilution on the activity of spermatozoa. *Brit. Jour. Exp. Biol.*, **5**: 337–344.

Gray, J., 1928b. The senescence of spermatozoa. *Brit. J. Exp. Biol.*, **5**: 345–361.

Hartmann, Max, 1940. Die stofflichen Grundlage der Befruchtung und Sexualität im Pflanzen- und Tierreich. I. Die Befruchtungsstoffe (Gamone) der Seeigel. *Die Naturw.*, **28**: 807–813.

Hartmann, Max, O. Schartau, and K. Wallenfels, 1940. Untersuchungen über die Befruchtungsstoffe der Seeigel. II. Gyno- und Androgamone des Seeigels Arbacia pustulosa. *Biol. Zent.*, **60**: 398–438.

Harvey, E. B., 1930. Effect of lack of oxygen on the sperm and unfertilized eggs of Arbacia punctulata, and on fertilization. *Biol. Bull.*, **58**: 288–292.

Hayashi, Teru, 1940. A relation between the dilution medium and the survival of spermatozoa of Arbacia punctulata. *Biol. Bull.*, **79**: 365.

Hayashi, Teru. Unpublished.

Henle, Werner, 1938. The specificity of some mammalian spermatozoa. *Jour. Immun.*, **34**: 325–336.

Henle, W., G. Henle, and L. A. Chambers, 1938. Studies on the antigenic structure of some mammalian spermatozoa. *Jour. Exp. Med.*, **68**: 335–352.

Just, E. E., 1922. Initiation of development in the eggs of Arbacia. I. Effect of hypertonic sea-water in producing membrane separation, cleavage, and top-swimming plutei. *Biol. Bull.,* **43**: 384–400.

Just, E. E., 1928. Initiation of development in Arbacia. V. The effect of slowly evaporating sea-water and its significance for the theory of auto-parthenogenesis. *Biol. Bull.,* **55**: 358–368.

Just, E. E., 1929a. Initiation of development in Arbacia. VI. The effect of sea-water precipitates with special reference to the nature of lipolysin. *Biol. Bull.,* **57**: 422–438.

Just, E. E., 1929b. The fertilization reaction in eggs of Paracentrotus and Echinus. *Biol. Bull.,* **57**: 326–331.

Just, E. E., 1939. Basic methods for experiments on eggs of marine animals. Philadelphia. P. Blakiston's Son and Co. 89 pp.

Lillie, F. R., 1913. The behavior of spermatozoa of Nereis and Arbacia with special reference to egg extractives. *Jour. Exp. Zoöl.,* **14**: 515–574.

Lillie, F. R., 1914. Studies of fertilization. VI. The mechanism of fertilization in Arbacia. *J. Exp. Zoöl.,* **16**: 523–590.

Lillie, F. R., 1915. Studies of fertilization. VII. Analysis of variations in the fertilizing power of sperm suspensions of Arbacia. *Biol. Bull.,* **28**: 229–251.

Lillie, F. R., 1919. Problems of fertilization. Chicago. The University of Chicago Press. 278 pp.

Mudd, E. B. H., S. Mudd, and A. K. Keltch, 1929. Effect of echinid egg waters on the surface potential difference of the sperm. *Proc. Soc. Biol. Med.,* **26**: 392–394.

Popa, G. T., 1927. The distribution of substances in the spermatozoön (Arbacia and Nereis). Studies by intra vitam stains and by stains of lipoids according to the methods of Schumacher. *Biol. Bull.,* **52**: 238–258.

Sampson, M. M., 1926. Sperm filtrates and dialyzates: Their action on ova of the same species. *Biol. Bull.,* **50**: 301–338.

Southwick, W. E., 1939. Activity-preventing and egg-sea-water neutralizing substances from spermatozoa of Echinometra subangularis. *Biol. Bull.,* **77**: 147–156.

Tyler, A., 1939a. Extraction of an egg-membrane-lysin from sperm of the Giant Keyhole Limpet. *Proc. Nat. Acad. Sci.,* **25**: 317–323.

Tyler, A., 1939b. Crystalline echinochrome and spinochrome: Their failure to stimulate the respiration of eggs and of sperm of Strongylocentrotus. *Proc. Nat. Acad. Sci.,* **25**: 523–528.

Tyler, A., and K. O'Melveny, 1941. The role of anti-fertilizin in the fertilization of sea urchin eggs. *Biol. Bull.,* **81**: 364–375.

Woodward, A. E., 1921. The parthenogenetic effect of echinoderm egg-secretions on the eggs of Nereis limbata. *Biol. Bull.,* **41**: 276–279.

HETEROCINETA PHORONOPSIDIS SP. NOV., A CILIATE FROM THE TENTACLES OF PHORONOPSIS VIRIDIS HILTON

EUGENE N. KOZLOFF

Department of Zoölogy, University of California, Berkeley

INTRODUCTION

The infestation of the tentacles of *Phoronopsis viridis* Hilton by a small ciliate of the family Ancistrocomidae Chatton and Lwoff[1] (order Holotricha, suborder Thigmotricha) was called to my attention by Professor Harold Kirby. A preliminary study of this ciliate, from slides prepared in his laboratory from material collected in Bodega Bay, California, in November, 1943, disclosed that on the basis of the organization of the ciliary system it appeared to be most closely related to species of the genus *Heterocineta* Mavrodiadi, ectoparasitic on fresh water mussels, prosobranchs, and pulmonates (Jarocki; 1934, 1935).

In June, 1945, I collected additional material of *Phoronopsis viridis*[2] in an intertidal mud flat in Tomales Bay. From observations of the living ciliates it was determined that this new species, which will be described herein as *Heterocineta phoronopsidis* sp. nov., differs fundamentally from other species of *Heterocineta* in having a groove-like depression originating on the left side of the body near the anterior end and extending posteriorly along the dorsal surface close to the left margin. I have studied a species of *Heterocineta* ectoparasitic on *Physa cooperi* Tryon from a locality near Mt. Eden, California, which agrees perfectly with the description of *Heterocineta janickii* given by Jarocki (1934). This ciliate, like *H. phoronopsidis,* has eight ciliary rows, but these are restricted to a more narrow area on the ventral surface. There is no dorso-lateral groove in *H. janickii.* In none of Jarocki's descriptions of ciliates of the genus *Heterocineta,* which apparently were based to a large extent upon living material, is there any mention of such a groove.

TECHNIQUE

For observation of the living ciliates the tentacles of *Phoronopsis viridis* were detached from the rest of the body by means of forceps and comminuted in a drop of sea-water on a slide. Fixation of the organisms for permanent preparations was accomplished by preparing smears in this manner on coverglasses and dropping them onto the surface of the fixative in a Petri dish. For a study of the general morphology, staining with iron hematoxylin gave good results on material fixed in Schaudinn's fluid. Differentiation of the ciliary system by impregnation with activated protein silver (protargol) was successful following fixation in Hollande's cupric-picro-formol mixture and Schaudinn's fluid, but this method was no more satisfactory than staining with iron hematoxylin. The Feulgen nuclear reaction was used after fixation in Schaudinn's fluid and a saturated aqueous solution of mercuric chloride with 5 per cent of glacial acetic acid.

---

[1] Chatton and Lwoff (1939) proposed the family Ancistrocomidae to include those ciliates formerly assigned to the family Hypocomidae Bütschli which differed from the type genus of the latter (*Hypocoma* Gruber) in having the suctorial tentacle disposed terminally rather than subterminally and the ciliary rows arranged singly rather than in pairs.

[2] Professor W. A. Hilton of Pomona College has kindly identified the phoronid species from Tomales Bay as *Phoronopsis viridis* Hilton (1930). It should here be noted, however, that no systematic revision of the phoronids from the Pacific Coast has been given in the literature and it is not impossible that *P. viridis* will later be shown to be identical with one of the species described earlier.

HETEROCINETA PHORONOPSIDIS sp. nov.

The body is elongated, asymmetrical, and flattened dorso-ventrally. Twenty living individuals taken at random ranged in length from 26 μ to 37 μ, in width from 11 μ to 16 μ, and in thickness from 6.5 μ to 11 μ, averaging about 29 μ by 14 μ by 8 μ. As seen in dorsal view (Fig. 1A) the left side of the ciliate is conspicuously rounded, while the right side is by comparison very little curved. The body is usually widest at a point a short distance behind the middle and is rounded posteriorly. The attenuated anterior end is deflected toward the left, truncate at the tip, and bent ventrally. The reduced ciliary system, to be described presently, is disposed in a shallow concavity occupying the anterior four-fifths of the ventral surface (Fig. 1B); the dorsal surface and that part of the ventral surface posterior to the ciliary area are convex.

A contractile suctorial tentacle enables the ciliate to attach itself to epithelial cells of the tentacles of the host and to feed upon their contents. When fully extended the suctorial tentacle of *Heterocineta phoronopsidis* is about 4 μ in length and 1.5 μ in diameter; it is contracted as soon as the ciliate is dissociated from the host and is seldom preserved in an extended condition in fixed individuals except those which have been fixed in a position of attachment to the host.

The internal tubular canal (Fig. 1, c) continuous with the suctorial tentacle is about 1.5 μ in diameter in its anterior portion, which is directed dorsally, and becomes abruptly narrower in its posterior portion, which is directed ventrally and obliquely to the right. In some living specimens and in suitable preparations stained with iron hematoxylin the canal can be traced along the right side of the body to a point a short distance posterior to the macronucleus.

The cilia of *Heterocineta phoronopsidis* are about 5 μ in length and markedly thigmotactic. They are disposed in eight longitudinal rows limited to the shallow concavity on the ventral surface (Fig. 1C). All eight rows originate near the base of the suctorial tentacle. Each of the first five rows from the right margin is about three-fifths the length of the body. The fourth and fifth rows are as a rule practically straight, while the outer three are appreciably curved. The remaining three rows become progressively longer and inflexed in such a way that they end one behind the other near the mid-line. The eighth and longest row terminates at a point about four-fifths the distance from the anterior end of the body to the posterior end. The cilia of the anterior part of the thigmotactic system move rather actively, those of the posterior part sluggishly.

The shallow groove-like depression which distinguishes *Heterocineta phoronopsidis* from other species of *Heterocineta* has its inception on the left side of the body near the anterior end and is about four-fifths the length of the body (Fig. 1A, g). As it extends posteriorly it comes to lie on the dorsal surface along the left margin.

The groove is visible only in living individuals. There are no traces of ciliature at any point along its course. Staining with iron hematoxylin and impregnation with protein silver fail to bring out any basal granules in the region occupied by the groove.

The cytoplasm is colorless and contains a number of small refractile granules in addition to food inclusions. The refractile granules (Fig. 1A, cg) are apparently lipoid droplets, as they are dissolved out by toluol used for clearing following staining. At least one large food-vacuole and usually several smaller ones are present near the posterior end of the body (Fig. 1, fv). The contents of the food-vacuoles are seen to consist mainly of ingested nuclei or fragments of nuclei from the epithelial cells of the tentacles of the host.

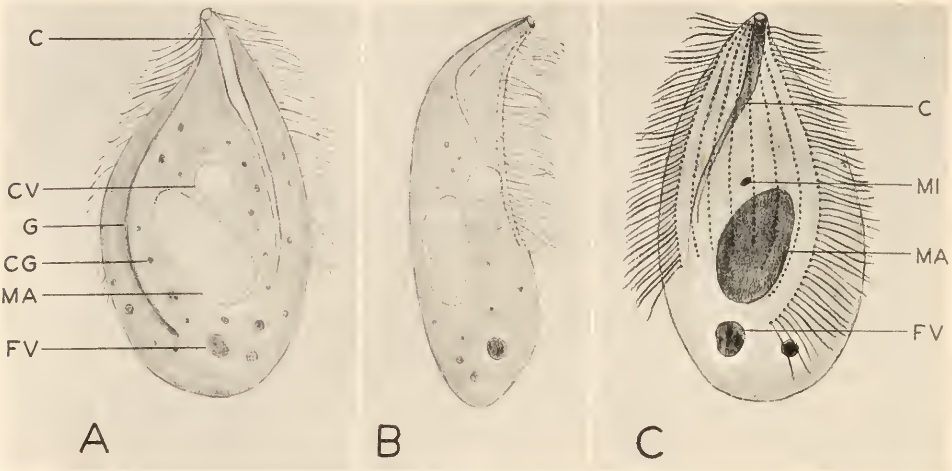


FIGURE 1. *Heterocineta phoronopsidis* sp. nov.

A. Dorsal aspect, from life; B. lateral aspect from right side, from life; C. ventral aspect. Schaudinn's fixative-iron hematoxylin. Drawn with aid of camera lucida. × 1940.

c = internal tubular canal, cg = cytoplasmic granule, cv = contractile vacuole, fv = food vacuole, g = dorso-lateral groove, ma = macronucleus, mi = micronucleus.

The contractile vacuole (Fig. 1, cv) lies near the middle of the body and opens to the exterior on the ciliated ventral surface. I have not distinguished a permanent opening in the pellicle.

The oval or rod-shaped macronucleus (Fig. 1, ma) is placed dorsally near the center of the body, its longitudinal axis lying obliquely to the longitudinal axis of the body. In ten individuals fixed in Schaudinn's fluid and stained by the Feulgen nuclear reaction on the macronucleus ranged in length from 5.25 μ to 7.5 μ and in width from 3 μ to 4.5 μ.

The fusiform, rod-shaped, or crescentic micronucleus (Fig. 1C, mi) is situated anterior to the macronucleus. It is very difficult to distinguish in living specimens and is stained only weakly by iron hematoxylin and the Feulgen nuclear reaction. In ten individuals fixed in Schaudinn's fluid and stained by the Feulgen reaction the micronucleus ranged in length from 1.5 μ to 2.25 μ and in width from 0.75 μ to 1.2 μ.

When attached to the tentacles of the host *Heterocineta phoronopsidis* is almost immobile, exhibiting only a passive vibratory motion due to the energetic movement of the epithelial cilia. When dissociated from the host the ciliate swims slowly, usually rotating on its longitudinal axis and tracing wide arcs with its attenuated anterior end.

Heterocineta phoronopsidis sp. nov.

Diagnosis: Length 26 μ–37 μ, average about 29 μ; width 11 μ–16 μ, average about 14 μ; thickness 6.5 μ–11 μ, average about 8 μ. The anterior end is attenuated, bent ventrally, and provided with a contractile suctorial tentacle continuous with an internal tubular canal. The ciliary rows are eight in number and originate near the base of the suctorial tentacle. The first five rows from the right are about three-fifths the length of the body, while the remaining three rows become progressively longer and are inflexed in such a way that they end one behind the other near the mid-line. A groove-like depression, without any trace of ciliature, extends from the anterior end of the body posteriorly along the dorsal surface close to the left margin. Ectoparasitic on the tentacles of *Phoronopsis viridis* Hilton (Tomales Bay, California). Syntypes are in the collection of the author.

LITERATURE CITED

Chatton, E., and A. Lwoff, 1939. Sur la systématique de la tribu des thigmotriches rhynchoïdés. Les deux familles des Hypocomidae Bütschli et des Ancistrocomidae n. fam. Les deux genres nouveaux Heterocoma et Parhypocoma. *C. R. Acad. Sci. Paris,* **209**: 429.

Hilton, W. A., 1930. Phoronida from the coast of southern California. *Jour. Ent. and Zool.,* **22**: 33.

Jarocki, J., 1934. Two new hypocomid ciliates, Heterocineta janickii sp. n. and H. lwoffi sp. n., ectoparasites of Physa fontinalis (L.) and Viviparus fasciatus Müller. *Mém. Acad. Cracovie, Cl. Sci. math. nat., B(II),* **1934**: 167.

Jarocki, J., 1935. Studies on ciliates from fresh-water molluscs. I. General remarks on protozoan parasites of Pulmonata. Transfer experiments with species of Heterocineta and Chaetogaster limnaei, their additional host. Some new hypocomid ciliates. *Bull. int. Acad. Cracovie, Cl. Sci. math. nat., B(II),* **1935**: 201.

MARINE BIOLOGICAL LABORATORY
LIBRARY
WOODS HOLE, MASS.

ABSTRACTS OF SCIENTIFIC PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY, SUMMER OF 1945

The role of bacteria in the excystment of the ciliate Didinium nasutum. C. D. Beers.

Resting cysts were obtained by the completion-culture method, viz., by preparing small cultures in spring water with paramecia as food. Most of the didinia in such cultures encysted upon exhaustion of the food supply. Such cysts never became active spontaneously, nor were they bacteria-free.

Distilled water, sugars, salts of plant acids, pH changes, and metabolites of Paramecium were ineffective in inducing excystment.

Timothy and lettuce infusions, and peptone and yeast-extract solutions induced 78–94 per cent excystment within 9–12 hours at 28° C. The tentative conclusion that these substances were effective excystment-inducing agents *per se* was soon negatived by the observation that at the time of excystment, bacteria (introduced with the cysts) were always flourishing in the media, which had been originally sterile. To test more adequately the effect of bacteria, these same four media were inoculated with wild bacteria from Paramecium cultures and incubated 18–24 hours. The bacterized media when tested on cysts induced 89–95 per cent excystment within 3–4 hours at 28° C. and thus produced a distinct acceleration effect.

The special effectiveness of bacterized peptone suggested an examination of the role of amino acids in excystment. Nine such acids were tested, singly and in mixtures, in buffered solution, but none yielded an accelerative effect. Only those acids (e.g., histidine, arginine, proline, methionine) and mixtures which supported bacterial growth induced excystment, and then only when bacteria were flourishing, i.e., after 9–12 hours. Acid mixtures previously inoculated with bacteria produced the usual accelerative effect. Hydrolyzed peptone behaved similarly.

Boiling the bacterized acid mixtures, or peptone solutions or hydrolyzates destroyed their effectiveness, which, however, could be restored by inoculation with bacteria.

The results indicate that excystment in Didinium is induced through the agency of bacterial action. Further studies are in progress to identify the effective bacteria, and to ascertain the chemical nature of the substances responsible for excystment.

Cytological studies in Culex. C. A. Berger and Sister Mary Grell.

Cells in the hind-gut of Culex ($2n = 6$) are diploid at the beginning of larval life and are highly polyploid at pupation. This polyploid condition arises by repeated chromosome reduplication within the resting nucleus. During metamorphosis these cells undergo mitotic division. The first division of a 16-ploid cell is described. Unique cytological features of this division are as follows. There are six groups of chromosomes each composed of eight sister chromosomes. Homologous groups are paired, relationally coiled and apparently have their spindle attachment regions fused. In early prophase the association of sister chromosomes is so close that the eight appear as one. As prophase contraction proceeds the eight sister chromonemata become evident and are seen to be relationally coiled in two's, in pairs of two's, etc. The spindle attachment region undergoes successive division in late prophase. At metaphase 48 chromosomes can be counted. Anaphase separation is regular and homologous or sister chromosomes pair as they move to the poles. This work can be interpreted as favoring the first part of Darlington's hypothesis, that chromosomes are attracted in pairs only, but gives no support to the second part of the hypothesis, that pairs of pairs repel.

Accelerating metamorphosis in the tunicate, Styela partita. Lloyd M. Bertholf.

C. Grave discovered that metamorphosis in tunicate larvae can be hastened by dozens of different substances, from complicated extracts of tunicate and vertebrate tissues down to simple salts of several heavy metals, added to sea-water. He concluded that such acceleration is caused by a poisoning of the larval action-system, so that the adult action-system takes over sooner than normally, and that the chief agent in this poisoning is copper.

To ascertain how specific the need for copper is, an effort was made to hasten metamorphosis by various substances in which copper is absent. Isotonic solutions of NaCl alone or in combination with other salts and with lactose and sucrose were first used. All these solutions brought about metamorphosis much sooner than in the controls, provided the larvae were about 4 hours old or older; if younger, the animals usually died before metamorphosis or shortly afterward.

It is possible, however, that the salts used contained a threshold amount of copper and other heavy metals as impurities. Hence distilled water alone was next used. This killed the animals after a few minutes of continuous exposure, but if larvae of about 2 hours or older were immersed in distilled water for only ½ to 2 minutes and then transferred to normal sea-water, metamorphosis was much hastened, and no deleterious effects resulted.

It seems, then, that the effect of copper is not specific, but that similar effects can be produced by other means, including the physical shock of a large change in osmotic pressure.

Oxidation-reduction studies on Penicillium notatum and other organisms. Matilda Moldenhauer Brooks.

Redox potential and pH measurements by means of the Coleman electrometer were made of the media in which *Penicillium notatum* and several other organisms were grown. Daily readings were taken for a period of several weeks. *Penicillium* was grown in corn steep medium. *Aspergillus flavus, Mycoderma, Torula utilis* and *Sacchromyces cervesiae* were grown in modifications of Czapek-Doz media. Sterile conditions were maintained.

It was found that rH values ($= 2 \text{ pH} + E_h/.03$) for *Penicillium* were 8.4 to 8.7. For purified penicillin (100,000 Oxford units) the rH was 8.7. For other organisms it was either higher or lower. In the case of *Penicillium,* the E_h value became very negative (-0.25) and the pH alkaline (8.5). No other organism studied had these characteristics.

When flasks were tightly stoppered, the rH values were similar to those obtaining in cultures to which KCN had been added. Growth was hindered when aerobes were used and not affected in the case of facultative anaerobes.

It is suggested that the therapeutic action of penicillin and related organisms depends upon the balance between E_h and pH in the blood, which these organisms produce. This factor makes it incompatible for such organisms as *Staphylococcus aureus,* for example, to exist.

Organization of the giant nerve fiber system in Neanthes virens. Theodore H. Bullock.

The presence of giant nerve fibers in certain polychaete annelids has been known on the basis of anatomical studies, but their function and functional organization have not been investigated. The group is especially suitable for such studies since its members present a great diversity of neural development; giant fibers are present in varying pattern in many species, absent in others; the group is large, and favorable species for laboratory study are common. A survey of the functional anatomy of the giant system in representative forms has been undertaken to the end of adding perspective to our picture of the evolution of the nervous system and with the hope of finding material for special studies of nervous physiology. The electrical signs of nervous activity were used as a tool for revealing the functional anatomy.

The present report will be confined to *Neanthes virens* (*Nereis virens*). When the nerve cord is directly stimulated by single shocks there is recorded from the nerve cord or from the mid-ventral line of the intact animal, in any other part of the worm, a pattern of large spikes, several orders of magnitude higher in voltage than the action currents representing spontaneous activity of the small fibers of the nerve cord. These large spikes have the properties of single fiber action currents. The first is the largest, has the lowest threshold, fatigues the slowest, and arrives at a time representing a minimum conduction rate (assuming no delays) of 5 meters/sec. Unlike the others it is not all or none, but all or half or none; two independent units are present conducting at just the same rate but separable by threshold and fatigue. The second spike is intermediate in height, threshold, fatigue, and rate (4.5 m/s) between each half of the first spike and the later ones. A small third spike at 2.5 m/s may be alone or followed by another like itself. This pattern is constant from specimen to specimen and may be regarded as characteristic of the species. One can expect anatomically at least four giant fibers or conducting units: a pair larger than the rest but identical in average diameter; a single unit, next in size; and one or two small but still "giant" units. This corresponds precisely with the known anatomy, there being a pair of large lateral fibers, a smaller median unpaired fiber, and a pair of still smaller medially placed fibers. The present technic can assure certain relations difficult to establish histologically. There is no anastomosis between the lateral fibers such as occurs in *Lumbricus;* the fibers are all independent conducting units, none being a necessary efferent or afferent connection of another; all the fibers are unpolarized, conducting equally well in both directions (although segmental macrosynapses like those in *Lumbricus* have been described). The sensory connections of each fiber can be inferred from responses to mechanical stimuli. The giants can each be fired through sense organs by local stimulation of the skin (a light tap or dropping water) within certain segmental levels; the head is not necessary. The median fiber (second spike with direct electrical stimulation) is fired by stimuli in the anterior quarter, approximately; the smallest, slowest giants by stimuli in the posterior three quarters and a region of overlap of a few segments occurs. The fast lateral giants can be fired from any level but require stronger stimuli (water dropping from a few cms. higher for example). The evidence suggests a function as mediators of startle responses to three classes of stimuli—weaker anterior, weaker posterior, and stronger at any level (differences in threshold in different levels exist for each fiber within this scheme). The two laterals usually fire together but in certain cases they can be separated.

The plan in general is very like that in *Lumbricus* although the two belong to different classes and many polychaetes with just as close a relation have no or very differently organized giant systems.

The displacement of terns by gulls at the Weepecket Islands. Sears Crowell.

The changes in population at the colony of Common and Roseate Terns at the Weepecket Islands are described for a period of twenty years. The colony attained, by 1931, a population of 3500 adult terns. During the past ten years this colony of terns has gradually been replaced by breeding Herring Gulls. The terns are probably incapable of successful reproductive activity if gulls are near, even though the latter do no direct injury to the terns.

The members of the Weepecket colony have been redistributed among other colonies of southern Massachusetts as shown by recoveries of banded birds.

Conditions favor a recolonization by the terns if the gulls are evicted or abandon the islands.

The influence of drugs on heat narcosis. A. Froehlich.

When the temperature of the surrounding water is slowly raised aquatic animals, such as crustaceae, fishes, tadpoles and frogs, show complete loss of voluntary and reflex muscular activity at a "critical point" of temperature which is characteristic for each species. This condition is reversible; transference into cool water causes the animals to recover promptly. "Heat narcosis" resembles narcosis brought about by drugs (alcohol, ether, etc.) in every way, except that where the former increases oxygen consumption, the drugs diminish oxygen consumption.

For reasons too numerous to mention here, I decided to investigate the influence of theophylline (as theophylline natrio-aceticum) on the "critical point" of heat narcosis. The experiments were performed on *Fundulus heteroclitus* at the M.B.L. in Woods Hole during the summer months of 1944 and 1945 and at the May Institute for Medical Research, Cincinnati, Ohio, on field frogs and tadpoles during the winter and spring of 1944–1945.

Theophylline given subcutaneously or intramuscularly in doses which had no visible effect on the behavior of the experimental animals produced a considerable lowering of the "critical point" in heat narcosis. The same effect was obtained if the animals were placed in a weak solution of theophylline.

Theophyllinized animals died much sooner than did controls if access to air was restricted. The water in which such animals died showed far greater acidity due to accumulation of CO_2.

Asphyxiation alone produced a lowering of the "critical point" similar to that obtained with theophylline.

Methylene blue (intramuscularly to Fundulus) produced effects on the "critical point," susceptibility to asphyxiation and acidity of the water which were similar to those obtained with theophylline.

In the experiments with theophylline as well as in those with methylene blue, previous conditioning in a 1 : 100,000 solution of quinine sulfate counteracted to a greater or a lesser degree the expected lowering of the "critical point."

It can be concluded that the action of theophylline and methylene blue on these experimental animals is, in part at least, to increase the demand for oxygen, and that quinine reverses this action by decreasing respiratory metabolism.

As I had previously found (with E. Zak) that an important part of the action of theophylline consists in increasing tissue permeability, I feel justified now in assuming that this phenomenon is caused by a condition of hypoxemia and acidosis (local asphyxia) in the tissues.

Reactions of oyster (Ostrea virginica) to free chlorine. Paul S. Galtsoff.

By measuring the rate of flow of water through the gills and by recording the shell movements it was possible to demonstrate that both the pumping mechanism of the oyster and its adductor muscle are very sensitive to free chlorine. In many oysters the first treatment with the concentrations as low as 0.01 or 0.02 p.p. million causes complete cessation of current and closure of shells, although there are specimens in which complete cessation of pumping and closing of shells takes place only in the concentrations approaching 0.5 p.p.m. Repeated treatments develop increased tolerance and pumping may be resumed at the concentrations much stronger than those which produced strong initial effect. Pumping, however, is not maintained at the concentrations of one p.p.m. or greater.

Variation in the sensitivity and development of tolerance are apparently associated with the secretion of mucus which provides protective coating for tentacles, mantle, and gills. Observations with a strobotac show that lateral cilia of the excised gill filaments continue to beat even at the concentration of 3 p.p.m. The cessation of pumping activity of an intact organism is due, therefore, not to the failure of the lateral cilia, but to the reaction of the regulatory mechanism of the pallium, which prevents the entrance of water to the gills, and to a certain extent to the disturbance of the rhythm of ciliary motion over the entire ciliated surface of the demibranches.

The presence of free Cl in water may materially impede the purification of oysters. It is therefore necessary that water, sterilized by chlorination and used in a process of purification, contains no residual Cl.

Development of granule-free fractions of Arbacia eggs. Ethel Browne Harvey.

A granule-free fraction of the *Arbacia punctulata* egg is obtained by breaking the egg with centrifugal force into two halves, and then breaking the lighter (white) half into two quarters, one of which contains all the remaining granules; and the other, the "clear quarter," is free of all granules visible in the living egg. This clear quarter contains the oil, nucleus, and most of the matrix or ground substance, but no mitochondria, yolk, or pigment. When fertilized, this clear quarter in many cases throws off a fertilization membrane, cleaves quite regularly, forms a perfect blastula and gastrula and pluteus. This pluteus may be quite normal with gut and skeleton, and later develops pigment spots, but is much clearer and less granular than that from the white half. It is approximately half the size of the pluteus from the white half and quarter the size of the pluteus from the whole egg. There is a considerable delay throughout development beginning with first cleavage, in spite of the fact that two nuclei (♂ and ♀) are present with a small amount of cytoplasm. The visible granules of the egg are therefore not necessary for development. The important substance in the cytoplasm is the ground substance or matrix, which is optically empty in the living state.

In vivo and in vitro glycogen utilization in the avian nematode Ascaridia galli. W. Malcolm Reid.

Glycogen constitutes one-third or more of the dry weight of many parasitic nematodes and flatworms. Extensive *in vitro* experiments upon glycogen utilization have been carried out by different investigators chiefly upon mammalian nematodes, cestodes, and trematodes. Von

Brand with *Ascaris lumbricoides* showed that 45 per cent of the glycogen reserve was utilized by females during 48 hours. Recent experiments upon fowl nematodes and cestodes have shown a much higher rate of glycogen utilization when the host had been starved for a short time. In a typical experiment with *Ascaridia galli,* 75 per cent of the glycogen reserve was utilized in 48 hours by female worms. With the fowl castode, *Raillictina cesticillus,* this reserve was depleted even more rapidly, 94 per cent of the glycogen being utilized in 24 hours.

Until a study using simultaneously *in vivo* and *in vitro* methods upon the same parasite has been completed, a comparison of the results of such experiments can have little meaning. Furthermore, such a study would serve as a check upon the earlier *in vitro* experiments which need re-examination now that improvements in technique have brought some of these results under question.

Glycogen determinations were made upon three groups of *A. galli.* Group I were controls and consisted of worms which were removed from the host after a normal feeding period. Group II worms were starved within the host for 48 hours before glycogen determinations were made. Group III consisted of parasites removed from the same hosts used for Group I, but these parasites were starved anaerobically for 48 hours at 41.5° ± 1° C. in one per cent saline using the same *in vitro* methods that were used on mammalian forms. Separate determinations were made on both males and females since sex differences in glycogen content were known to exist. The mean glycogen content for approximately ten samples for each group expressed in per cent of the wet weight of the worms is as follows: Group I females, 4.66; Group II females, 1.16; Group III females, 1.01; Group I males, 3.81; Group II males, 0.43; and Group III males, 0.26. The similarity in the rate of glycogen utilization with both males and females under the two conditions probably indicates that the *in vitro* methods used by early investigators reflect reliable information about normal glycogen metabolism within the host. Comparison between the glycogen utilization in the avian *A. galli* with the mammalian *A. lumbricoides* indicates that the much higher utilization rate in *A. galli* is real and not due to differences in technique.

Balanced centerwell solutions for manometric experimentation with cyanide. W. A. Robbie.

It has been demonstrated, both experimentally and theoretically, that the potassium cyanide-potassium hydroxide absorption solutions recommended by Krebs (1935, *Biochem. Journ.*, **29**: 1620) are not in hydrogen cyanide equilibrium with the experimental fluids for which they were designed. It is possible, however, to prepare, on the basis of experimental determinations, potassium cyanide-potassium hydroxide mixtures which will absorb carbon dioxide and maintain hydrogen cyanide equilibria with cyanide solutions of 0.011 M or less. The hydrogen cyanide tension of calcium cyanide solutions saturated with calcium hydroxide varies only with the concentration of the calcium cyanide and the temperature. This type of centerwell mixture will absorb carbon dioxide effectively and maintain equilibrium with hydrogen cyanide solutions up to 0.01 M.

*Studies of the muscle twitch recorded by electronic methods.** Alexander Sandow.

Piezoelectric, cathode-ray oscillographic methods have been devised for recording the various mechanical changes of the isometric twitch of skeletal muscles. To register the latency relaxation, LR (the minute precontractile elongation of a stimulated muscle during the latter half of the latent period), the apparatus is used, in effect, as an electronic lever which converts the LR into a 500,000 × magnified deflection on the cathode-ray screen. The piezoelectric pulse corresponding to the main contraction and relaxation periods is electronically differentiated and thus at each instant the cathode-ray deflection for this record is proportional to the rate of tension change in the course of the twitch.

These methods have been used to study the effect of maximal tetani of lengths from ⅕ to 10 sec. on the mechanical features of the twitch of the frog sartorius. The results prove that the separate processes that underlie the LR, the use of tension, and the post-contractile relaxation, are each uniquely affected by the tetanic activity. E.g., a 2 sec. tetanus causes a 10 per cent increase in the maximum rate of tension rise in a twitch, but a 40–60 per cent increase in the maximum rate of relaxation. The great lability of the relaxation process associated with the new chemical environment induced by the activity is specially significant in indicating that relaxation is not passive but is chemically driven.

---

* Supported in part by a grant from the Penrose Fund of the American Philosophical Society.

The LR shows certain temporal features like those of Brown's alpha-process, thus indicating that it is an external mechanical sign of the alpha-process. Detailed analysis of the effect of activity and of pH on the LR, especially in reference to the duration of the latent period, suggests that the latent period is an interval during which myosin-ATPase is splitting ATP, and leads to the inference, now being subject to further test, that the LR corresponds to the formation of an enzyme-substrate complex between myosin and ATP which provides a mechanism for directly energizing and activating the myosin for contraction.

Experimentally induced tumors in an insect. Berta Scharrer.

In *Leucophaea maderae,* a large Orthopteran, the recurrent nerve was cut at various levels. This nerve, which belongs to the stomatogastric nervous system, innervates the anterior portion of the alimentary canal as well as the salivary glands and their reservoir. Within ten days to several months after the operations tumors developed in organs innervated by the recurrent nerve. Frequent sites of tumorous growth were the anterior portion of the mid-gut and the salivary reservoir. In the fore-gut and in the salivary glands well developed tumors were relatively rare. Several types of control operations, such as allatectomy and castration in which the recurrent nerve had remained intact, did not cause the development of tumors. Some of the tumors obtained after the cutting of the recurrent nerve attained considerable sizes. Histologically they consist of layers of cells which show various degrees of abnormality. In advanced stages part of the cells break down into a debris of brown color. About 300 specimens, nymphs as well as male and female adults, with experimental tumors, were studied.

The origin of neurosecretory granules from basophil constituents of the nerve cells in fishes. Ernest Scharrer.

Neurosecretory granules do not appear to be formed in association with the Golgi apparatus or the mitochondria, but with the basophil constituents of the secreting nerve cells. Three modes of origin of the granules have been observed. In the preoptic nucleus of most fishes the granules originate in association with the peripherally located Nissl bodies. The latter diminish to the extent to which the acidophil neurosecretory granules increase. In a second type found in the preoptic nucleus of Ameiurus, Noturus, Centropristes, and others the nuclei of the secreting nerve cells show invaginations. These are filled with basophil cytoplasm which may contain acidophil granules. In a third type which is characteristic of the nucleus lateralis tuberis of catfishes, the acidophil granules originate within the nuclei of the cells, apparently at the expense of the nuclear chromatin. All three types may occur in the preoptic nucleus of Centropristes.

*Evidence of a metabolic effect by potassium in lowering the injury potential of invertebrate nerve.** Abraham M. Shanes.

The action of potassium on the injury potential of spider and blue crab nerve has been studied over a concentration range of one to 530 mM. When the magnitude of these potentials is plotted against the logarithm of potassium concentration, the relative effectiveness of low potassium concentrations in lowering the potential is found to be ½ that of concentrations above 30 to 40 mM. The data may be replotted on a log-log graph on the assumption that potassium is inactivating an enzyme, the active form of which is proportional to the resting potential. Two straight lines intersecting at 40 mM fit the data very well, the slope at lower concentrations being about ½ and at higher concentrations about one. This graph is like one which has been obtained for the effect of urethane on oxygen consumption in yeast and Arbacia; in this case the inhibitor is believed to act on two processes. The same interpretation may be applied to potassium.

---

* Aided in part by a grant from the American Academy of Arts and Sciences.

The similarity of potassium to an actual inhibitor is even stronger if consideration is given to the effect on activity. Only at a concentration corresponding to almost complete cessation of the process affected at low concentration does activity appreciably and suddenly decrease. Thus, in crab nerve, conduction ceases between 37 and 42 mM. Potassium and excitability is unaffected up to 37 mM.

The effect of low potassium concentrations is definitely correlated with the simultaneous inhibition of an aerobic metabolic process which supports the injury potential. In concentrations of 10 to 30 mM potassium eliminates ⅔ of this process—values corresponding closely to those obtained previously in frog nerve.

*Physical-chemical studies on chromosomal nucleoproteins.** Kurt G. Stern.

The object of this research is to determine the size and shape of desoxyribosenucleoproteins, isolated from cell nuclei, with the aid of such quantitative methods as ultracentrifugation, diffusion, electrophoresis, viscosity, x-ray diffraction, and similar techniques. In this cooperative study, S. Davis, P. Macaluso, S. C. Shen, and I. Fankuchen are collaborating with the writer.

Thus far, the desoxyribosenucleoproteins from the nuclei of chicken red blood cells and from calf thymus gland have been studied. Measurements in the analytical ultracentrifuge, in the diffusion apparatus, and in Ostwald viscometers, performed on solutions of these purified nucleoproteins in one M. NaCl, indicate a molecular weight of the order of two to three million and axial ratios varying from 35 : 1 to 100 : 1. The discrepancy of the results obtained with independent techniques casts considerable doubt on the suitability of this solvent, proposed by Mirsky and Pollister, with regard to the native state of the nucleoproteins. It appears that these conjugated proteins are appreciably dissociated in M.NaCl-solution. According to preliminary experiments, one M.glycine appears to be a solvent better suited for physical-chemical studies on these macromolecules.

The theory that these desoxyribosenucleoproteins are capable of assuming a more or less *helical shape* in solution as a function of the nature and ionic strength of the solvent, is advanced as a working hypothesis. Thus it is assumed that these molecules reflect in their configuration, on a molecular scale, the coiling and uncoiling of the chromosomes of which they represent important constituents. Plastic models, constructed in accordance with this hypothesis, were demonstrated at the Seminar.

Action of quitenine on the livers of tautog and toadfish. Charles H. Taft.

When quinine is treated with potassium permanganate the vinyl group is oxidized to a carboxyl group yielding quitenine.

It has been shown (Dauber, M., 1920; *Zeit. für Expt. Path. u. Therapie,* **21**: 311) that quitenine had a damaging action on kidney tubules. Taft and Place (1944; *Texas Reports on Biol. and Med.,* **2**: 61) showed that quitenine was more injurious to the kidneys of a glomerular fish than to the kidneys of an aglomerular fish.

Quitenine dihydrochloride in a 0.25 molar solution was injected subcutaneously into the side of the fish. The doses used were 1, 2, and 4 mM/Kg. Fish were killed by a blow on the head after varying intervals of time. The livers were placed in Bouin solution. Sections were cut 6 μ thick.

On gross examination a few tautog livers were abnormally soft. Gall bladder was a greenish blue in all cases. In the toadfish the liver was soft in a few cases. Color of gall bladder ranged from white through pale pink, orange, yellow green to green. Variation in color is probably due to variation in amount of bile pigment production or to oxidation of bile pigment. The von Kupffer cells were undamaged as were pancreatic cells of hepatopancreas.

Microscopic examination of toadfish liver shows fatty metamorphosis and some parenchymatous degeneration. Microscopic examination of the tautog liver showed fatty metamorphosis, albuminous degeneration, hydropic degeneration, and parenchymatous degeneration.

Quitenine is more damaging to the liver of the tautog than to the liver of the toadfish. The damage to the livers is not as severe as it was in the kidneys.

---

* This work was made possible by a grant of The Carrie S. Scheuer Foundation of New York.

Differences in sensitivity, hatchability curves, and cytological effects between Habrobracon eggs x-rayed in first meiotic prophase and metaphase. Anna R. Whiting.

Unlaid Habrobracon eggs were x-rayed in first meiotic prophase (diplotene) and in late metaphase and allowed to develop parthenogenetically. Those treated in prophase have 50 per cent hatchability at about 12,000 r (lethal dose about 45,000 r); give an exponential hatchability curve which tends to become linear when dose is fractionated; may show, after treatment, fragments or bridges or both in division I, in division II or in both. Those treated in late metaphase have 50 per cent hatchability at about 400 r (lethal dose about 2,000 r); give a linear hatchability curve which does not change with fractionation of dose; may show fragments but no bridges in division I, either or both in division II. All eggs treated in either stage with lethal dose develop at least to first cleavage (20 per cent continue to blastoderm); show bridges and sometimes fragments in cleavage. A correlation of chromosome form, movement, and tension at time of treatment with sensitivity and cytological effects exists which suggests that x-ray injury is due to direct "hits" on chromosomes, and that sensitivity is associated with degree of tension to which chromosomes are exposed during irradiation; that nature of chromosome changes is due to their form and proximity during treatment. Lethal dose is not lethal to the treated cell (oocyte) but to its descendents (embryo) since chromosome fragmentation is not lethal, loss of fragments is.

The problem of reversal of male haploidy by selection. P. W. Whiting.

Except for the almost sterile, highly inviable diploid males of the wasp Habrobracon obtained in experimental cultures, diploid males are unknown in the Hymenoptera, as also in rotifers, thrips, mites except Mesostigmata, aleurodids and iceryine coccids, and in the beetle Micromalthus. It is probable that all normal males in these groups are haploid and that male haploidy has been attained in an evolutionary sense not more than six or seven known times. One of these attainments, taking place in an ancestral hymenopteron probably in the early Jurassic, has come to involve the entire order. Three conditions characterize male haploidy: (1) Production of males from reduced unfertilized eggs. (2) Reduction or omission of meiosis in spermatogenesis. (3) Complementary sex determination with heterozygous "double dominant" females. The problem of reversal of male haploidy is not to attempt to re-integrate any Jurassic protohymenopteran species, but rather to obtain by methods of genetics a strain of *Habrobracon juglandis* with normal biparentalism of males as well as of females. Inbreeding gives diploid males homozygous for sex. Selection has increased their viability from one to sixty per cent as compared with females. Cell size of diploid males is abnormally large, but is reduced somewhat in strains of high viability. Spermatogenesis of diploid males is of the haploid type, lacking chromosome synapsis and resulting in diploid sperm. If a strain with chromosome synapsis can be derived, it is considered that the problem can be solved, since sex determination should then shift from the complementary to the back-cross type with digametic females.

Endomitosis in plants. E. R. Witkus.

The process called endomitosis was discovered by Geitler in 1939 in insect material. During this process there is a chromosomal reduplication without a nuclear division, no spindle is present and there is no true anaphase movement of chromosomes. Throughout the whole process the nuclear membrane remains intact. Geitler divided the process into four stages, which he termed endoprophase, endometaphase, endoanaphase, and endotelophase. During endoprophase the chromosomes become shorter and thicker. The stage at which the chromosomes have reached their highest degree of contraction is called endometaphase. The nuclear membrane is intact and the chromosomes are not aligned on an equatorial plate. The SA-region of the chromosomes divides and the chromatids or now endoanaphase chromosomes slightly separate. After this separation the chromosomes undergo reversion to the resting stage. This reversion process occurs during endotelophase. The resulting cell then is tetraploid.

This process was also found to occur in the tapetal cells of *Spinacia oleracea* (Spinach) and apparently this is the first time that endomitosis, as defined by Geitler, has been reported for plant material.

The tapetal cells of *Spinacia* undergo two successive divisions during the early prophase stages of meiosis. The first division is an incomplete mitotic division resulting in binucleate cells or in cells having dumb-bell shaped nuclei. The second of these divisions is in all cases endomitotic.

It becomes increasingly apparent that polyploidy brought about by a chromosomal reduplication without a nuclear division is of quite common occurrence in both plant and animal material. Endomitosis is only one of three known methods by which this can occur, although it has often been confused with all three in recent cytological literature. The first method is by a repeated reduplication in the resting nucleus as illustrated in the multiple complex cells of mosquito. The second is simply by a double reduplication in the resting nucleus as shown by certain cells in the root tips of polysomatic plants such as *Spinacia*. The third is by endomitosis.

It is interesting also to note that polyploidy arises by different methods in the root tip and tapetal cells of *Spinacia oleracea*.

A tetrahedral framework for native proteins? Dorothy Wrinch.

It was suggested in 1936 that a fabric or atomic lamina is an essential element in the structure of native proteins and the lactim cyclol fabric was formulated as a working hypothesis (*Nature,* **137**: 411). Today four different types of fabric—all necessarily polypeptide fabrics—are open for discussion; the lactim and enol cyclol fabrics, the hydrogen-bridged linear polypeptide fabrics, and the fabrics in which cyclized polypeptides are interlocked by hydrogen bridges (Jordan Lloyd and Wrinch, 1936; *Nature,* **138**: 758; Astbury and Wrinch, *ibid.,* **139**: 798; Wrinch, 1940; *Phil. Mag.,* **30**: 64; 1941, **31**: 177). This idea of an atomic lamina or fabric as characteristic of native proteins has been widely accepted and adopted. It has now been used to interpret the x-ray intensities in a study of horse methemoglobin in crystalline form.

We wish now to suggest that these (hol) intensities (Perutz, 1942; *Nature,* **150**: 324) suggest not only laminae parallel to the c-planes (Boyes-Watson and Perutz, 1943; *Nature,* **151**: 714) but also a second set of laminae at approximately the tetrahedral angle to the first. For this and a number of other reasons, the hypothesis is put forward that the native protein unit (of which there may be one, two, or more in the native protein particle) is built on a tetrahedral framework, the possibility that the enveloping truncated tetrahedron of the framework may be an octahedron not being excluded. In the case of horse methemoglobin, this suggestion implies trigonality of the individual frameworks about an axis approximately normal to the c-planes; thus it offers an interpretation of the fact that the lattice points and c-face centers in these planes form a triangular network which is very nearly equilateral. The hypothesis appears to bear very closely upon the fact that twins, trillings, and other compound crystals are extremely common in many different hemoglobins (Reichert and Brown, 1909; The Crystallography of the Hemoglobins, Washington, D. C.). This we propose for discussion the possibility that such situations as the apposition of tetrahedral frameworks by displacements plus rotations of (1) ⅓, ⅔ about the (111) axis; or (2) ⅙, ½, ⅚ about the (111) axis; or (3) ½ about the $(\bar{1}10)$ or $(11\bar{2})$ axes, or ¼, ½, ¾ about the (100) axes, etc., are here realized. Attention is also directed to the obvious manner in which this postulate lends itself to the interpretation of the space groups and classes of crystal symmetries found in x-ray (Fankuchen, 1941; *Ann. N. Y. Acad. Sci.,* **41**: 157) or classical studies of the native proteins.

Vol. 89, No. 3 December, 1945

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

NATURAL HETEROAGGLUTININS IN THE SERUM OF THE SPINY LOBSTER, PANULIRUS INTERRUPTUS. II. CHEMICAL AND ANTIGENIC RELATION TO BLOOD PROTEINS[1]

ALBERT TYLER AND BRADLEY T. SCHEER

William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena

In a previous report (Tyler and Metz, 1945) it has been shown that lobster-serum contains at least ten heteroagglutinins for sperm or blood cells of various animals. Each of the heteroagglutinins was found to act on all the species tested that belong to the same group of animals. Since the group, in most instances, represents a taxonomic class, the heteroagglutinins are termed class-specific. The heteroagglutinins were found to be most probably protein, and by means of electrophoresis they were shown to be distinct from the hemocyanin which Allison and Cole (1940) and Clark and Burnet (1942) had considered to be the sole protein present in lobster-serum.

The relatively small amount found to be present accounts for Allison and Cole's conclusion which was based on approximate identity of the copper to protein nitrogen ratios of purified hemocyanin and of whole serum. Clark and Burnet's evidence was actually to the effect that there is no protein present with active antigenic properties different from that of pure hemocyanin. This is in accord with the results obtained with antisera prepared against heteroagglutinin by injecting rabbits with agglutinin that had been absorbed on rabbit cells. In the present paper a precipitation method for preparing the heteroagglutinins free of hemocyanin is described, and results of an electrophoretic examination of the material are presented. The agglutinating action of fibrinogen preparations from plasma and further serological tests are also reported.

MATERIAL AND METHODS

Blood is quite easily obtainable from lobsters by means of a syringe inserted, between cephalothorax and abdomen, into the pericardial chamber. A twelve-inch lobster yields, in this manner, about 20 to 30 ml. of blood. For serum the blood was generally defibrinated by shaking with glass beads, filtered, and centrifuged; or it was occasionally allowed to clot, forced through a fine mesh wire screen, and centrifuged. For plasma the blood was drawn into a small amount of sodium

---

[1] This work has been aided by grants from the American Philosophical Society and the Rockefeller Foundation.

citrate solution, then subsequently filtered, centrifuged, and dialyzed against saline. One volume of 10 per cent citrate suffices to prevent fibrin-clotting in about 30 volumes of blood.

The agglutinative tests were made as previously described (Tyler and Metz, 1945) by mixing equal volumes of the sperm or blood cells (of sea-urchin, sheep, or other animal) and of serial two-fold dilutions of the test-solution adjusted to the appropriate salinity. Deviations from these proportions are specified in the tests.

Experimental Part

Separation of heteroagglutinins from hemocyanin by isoelectric precipitation

Hemocyanin was prepared from serum by isoelectric precipitation essentially as described by Allison and Cole (1940) and by Rawlinson (1940). This consists in dialysis against distilled water and then against dilute acetate buffer at the pH of the isoelectric point. Further purification is obtained by repeated solution in dilute ammonia and reprecipitation, by addition of acetate buffer (0.1 M., pH 4.5).

Rawlinson (1940), in the course of purification of hemocyanin from the *plasma* of the Australian spiny lobster, noted the presence of small amounts of protein which he considered to be fibrinogen. Such a non-hemocyanin protein is obtainable from the *serum* of the California spiny lobster, *Panulirus interruptus*.

When samples of serum or plasma of *Panulirus* were dialyzed against dilute, pH 4.5, acetate buffer, there invariably appeared small amounts of a pale precipitate that separated before the hemocyanin started to come down. The precipitates ranged in color from white to pink. After centrifugation, washing with distilled water and solution in dilute ammonia, the material was reprecipitated by slow addition of 0.01 M., pH 4.5 acetate buffer. The material was regularly found to start to precipitate at pH 5.0 and reach a maximum at pH 4.8. From the supernatants of the first precipitates the blue-colored hemocyanin was precipitated by continuation of the dialysis against the pH 4.5 buffer. The hemocyanin was obtained in crystalline form from concentrated solutions of it in dilute ammonia by the slow addition of dilute acetate buffer. Its precipitation was found to begin at pH 4.6 and to be complete at 4.5 to 4.4.

Samples of the purified hemocyanin and of the pale precipitate were tested for their ability to agglutinate the sperm or blood cells of various animals. After adjustment of the solution to appropriate pH and salinity by dialysis, they were tested on one per cent suspensions of the sperm of the polychaet, *Chaetopterus variopedatus;* the sea cucumber, *Stichopus californicus;* the starfish, *Pisaster ochraceus*; the sea-urchin, *Strongylocentrotus purpuratus*; the sea-squirt, *Ciona intestinalis;* and the grunion (smelt), *Leuristhes tenuis;* and of the erythrocytes of the sand bass, *Paralabrax maculatofasciatus;* the frog, *Rana pipiens;* the chuckwalla, *Sauromalus ater;* the pigeon, and sheep. The hemocyanin preparations, containing this material in amounts as great as or greater than normally present in the serum, were found to be completely inactive. The preparations of the pale, pH 4.8-5.0, precipitate gave very good agglutination of the cells of all the above named species.

Titer determinations were made with one of these preparations on sperm of *Strongylocentrotus*. In this case 0.2 ml. of serial two-fold dilutions of the solution were mixed with one drop of 10 per cent sperm-suspension. The protein concentration (from Kjeldahl nitrogen determination) of the solution was 0.7 per cent and its titer (minimum dilution giving definite microscopic agglutination) was 128. A sample of serum containing 5 per cent protein gave at the same time a titer of 256. This preparation showed, then, about 3½ times the activity of the whole serum.

Electrophoretic examination of the pale precipitate[2]

Another sample of the material freed of hemocyanin was reprecipitated at pH 5, dissolved in dilute ammonia, and dialyzed for 2 days in the cold against barbiturate buffer ($\mu = 0.05$) at pH 7.7. It was then examined electrophoretically in the

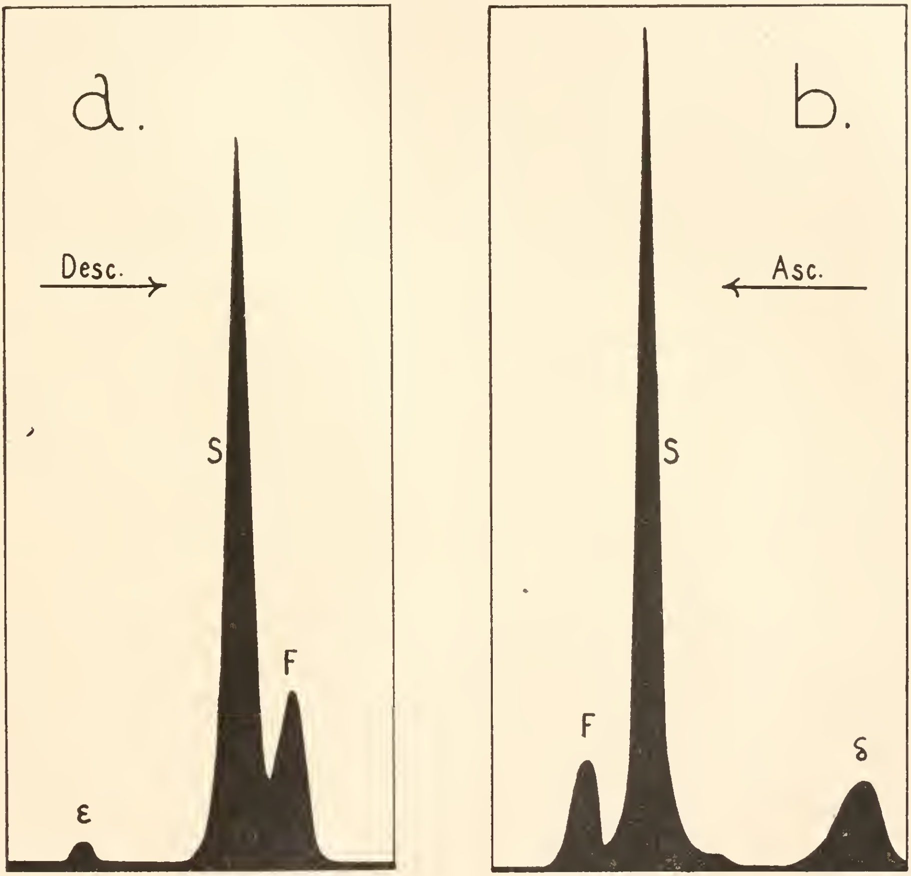


FIGURE 1. Electrophoretic patterns of pale (pH 5) precipitate from lobster-serum. *a*, descending (desc.) side; b, ascending (asc.) side; after 59 minutes of electrophoresis at pH 7.7, ionic strength 0.05 and 14.8 ma. Arrows show direction of migration. See text for further description.

---

[2] The apparatus employed was that constructed in the Division of Chemistry by Dr. Stanley M. Swingle to whom we are indebted for the electrophoresis of this material.

Tiselius' (1937) apparatus using the scanning method of Longsworth (1939). After 59 minutes of electrophoresis with a current of 14.8 ma., the patterns shown in Figure 1 were obtained. As may be seen from the figure two components, besides the δ- or ε-boundaries, are present in the serum. From the relative areas covered by the peaks the ratio of amount of slow component to that of fast component is approximately 5:1. At the end of the run the fast moving component was removed from the ascending side and the slow component (plus δ), from the descending side of the electrophoresis cell. After dialysis against normal saline, determinations were made of their agglutinative titers for rabbit cells and of the Kjeldahl nitrogen content. Samples of the original solution of the pale precipitate (taken from the cell after the run) and of normal lobster-serum were tested at the same time. The results are given in Table I. The nitrogen content of the solutions does not represent the relative concentrations of the components present in the original solution since there was some dilution with buffer upon their removal from the electrophoresis cell. As may be seen in Table I, the solution of the fast component showed no agglutinative activity for rabbit-erythrocytes although its nitrogen content was about one-third that of the slow component. The slow component proved highly active, giving almost twice the titer (per mg. N. content of solution) of the original solution and 24 times that of whole serum. This is approximately the order of magnitude of activity obtained (Tyler and Metz, 1945) for the components isolated by electrophoresis from whole serum.

TABLE I

Agglutinative titers of components obtained by electrophoresis of the pale (pH 5) precipitate from lobster-serum

Material	mg. Kjeldahl N. per ml.	Agglutinative titer on 1% rabbit cells	Titer/mg. N.
Fast component (F of Figure 1)	0.29	0	0
Slow component (S of Figure 1)	1.008	128	128
Original solution (from cell-residue)	3.85	256	66.5
Whole serum	11.97	64	5.3

The slow component obtained here was also tested on cells of all the animals listed on page 194, with the exception of *Sauromalus* and *Leuristhes*. It proved to be highly active with all of them. In the previous report lobster-serum was shown to contain at least ten "class-specific" heteroagglutinins. It is evident from the present results that these are represented by a single electrophoretic component of the serum, unless there is some active component in the stationary δ- or ε-boundary. The latter is, however, highly unlikely since the original material for the present test was obtained by precipitation at pH 4.8 to 5.0 and the electrophoresis was run at pH 7.7. For any material to remain in these stationary boundaries it would have to be isoelectric at the latter pH.

Preparation of fibrinogen and tests for heteroagglutinating activity

Lobster-plasma upon being brought to 25 per cent saturation with ammonium sulfate formed a white to pink precipitate which separated easily upon centrifugation. The precipitate was washed with distilled water and dissolved in sea water. Addition of fresh lobster-blood-cells to the solution caused it to form a firm clot. A pH 5.0 precipitate obtained directly from plasma was found to contain fibrinogen, which could be separated from the remaining protein material by precipitation with ammonium sulfate. None of the prepartions from serum were found to contain fibrinogen.

TABLE II

Agglutinative titers of protein preparations from plasma and serum

Material	mg. Kjeldahl N. per ml.	Agglutinative titer on *Strongylocentrotus* sperm	Titer per mg. N.
Fibrinogen preparation (I)	1.25	32 to 64	26 to 51
Pale precipitate (II)	1.25	64 to 128	51 to 102
Hemocyanin	7.4	0	0
Whole serum	8.5	128 to 256	15 to 30
Plasma	8.5	256 to 512	30 to 60

A fibrinogen preparation (I) was obtained from whole plasma by 25 per cent saturation with ammonium sulfate. The precipitate was dissolved and reprecipitated by dialysis to pH 5.0. This preparation was tested for agglutinating action on sperm of *Strongylocentrotus* in the same manner as on page 194. The supernatant from the 25 per cent ammonium sulfate precipitate was dialyzed against tap water and then brought to approximately pH 5 by dialysis against pH 4.5 buffer. This gave a pale precipitate (II) which resembled the pale precipitate from serum. After solution and dialysis against sea water it, too, was tested for agglutinating activity. The results are given in Table II along with simultaneous tests of whole serum, plasma, and hemocyanin. The presence of calcium in the sperm suspension does not interfere with the tests, since clotting of the fibrinogen does not occur unless fresh lobster-blood-cells are added. As the table shows, plasma has about twice the agglutinating activity of serum. The fibrinogen preparation proved about half as active as the pale precipitate.

Another pale precipitate was also obtained directly from plasma by dialysis against pH 4.5 buffer. When the precipitate was dissolved and brought to 25 per cent saturation with ammonium sulfate there separated out some material that proved to be fibrinogen. It appears from the experiments reported above that the isoelectric point of fibrinogen is not greatly different from that of the heteroagglutinin found in serum. This conclusion was verified by Mr. Maurice Rapport, who repeated some of our experiments, and made an electrophoretic examination of plasma and of protein preparations separated from plasma. The pH 5.0 precipitate from plasma showed two electrophoretic components, the patterns being similar to those of Figure 1. The smaller, faster component probably corresponded to the fast component observed in serum preparations. The other component, containing agglutinating activity, could not be separated further during 100 minutes of electrophoresis at pH 7.3, 1.2° C. and 20 ma.

Precipitation of the pH 5.0 precipitate from plasma with ammonium sulfate at 40 per cent of saturation removed nearly all of the agglutinating activity, but left behind a small amount of protein material. The ammonium sulfate precipitate contained 3.5 mg. Kjeldahl N./ml, and had a titer of 64 against *Strongylocentrotus* sperm. The supernatant contained 1.6 mg. N./ml, and titrated only to 4. Mr. Rapport showed that this small residue migrated rapidly in the electrophoresis apparatus at pH 7.3. It probably corresponded to the fast component from serum.

In the absence of more exhaustive chemical and electrophoretic separations it is not possible to decide with certainty whether the agglutinative activity found in fibrinogen preparations is associated with fibrinogen itself, or is due to the presence in these preparations of the heteroagglutinin fraction which is present in serum.

Antigenic relationship of the blood proteins

Two rabbits that were each given two courses of intravenous and intra-abdominal injections with a total of 375 mg. of purified hemocyanin produced very good precipitating antisera. The titers (end point of precipitation on mixing equal volumes of antiserum and serial dilutions of a 10 per cent hemocyanin solution) ranged from 10,000 to 20,000 in terms of antigen dilution and optimal proportions (second optimum, see below) were obtained at approximately one volume of 10 per cent hemocyanin to 10 to 20 volumes of antiserum. The antisera also reacted very well with whole lobster-serum, the optimal proportions point being about 9 volumes of antiserum to one volume of the lobster-serum.

Tests were then made of the ability of antiserum vs. hemocyanin to remove natural heteroagglutinin from whole lobster-serum. One volume of lobster-serum was absorbed with 9 volumes of the rabbit antiserum and the supernatant tested for ability to agglutinate rabbit-erythrocytes and *Strongylocentrotus* sperm. The absorbed serum gave no reaction with these cells, while control lobster-serum gave good agglutination out to dilutions of 1/90 (+ + + reaction) with the rabbit cells and 1/80 (+ reaction) with the *Strongylocentrotus* cells respectively.

It appears, then, that antibodies prepared against hemocyanin also react with the natural heteroagglutinins present in lobster-serum.

One of the antihemocyanin rabbit sera was also titrated with the solution of electrophoretically purified heteroagglutinin (slow component). A titer (dilution of antigen) of 128 was obtained for this solution which contained one mg. Kjeldahl N. per ml. A control hemocyanin solution containing 8 mg. N. gave a minimum titer (end point not reached) of 4096, or 512 per mg. N.

Another antiserum against hemocyanin was also titrated with various protein fractions separated from lobster-blood. The titer (dilution of antigen) of reprecipitated hemocyanin was 20,000 for a solution containing 6.6 mg. Kjeldahl N. per ml. or 3000 per mg. N. For the heteroagglutinin (pH 5 precipitate from serum, reprecipitated), the titer was 200 for a solution containing 1.6 mg. N. per ml. or 125 per mg. N. For the fibrinogen (ammonium sulfate precipitate from plasma), the titer was 200 for 3.4 mg. N. per ml. or 60 per mg. N.

In these titrations, it was sometimes noted that precipitation occurred in the first few tubes, containing concentrated antigen solutions. In intermediate dilutions, no precipitation occurred, but a second zone of precipitation appeared in the higher dilutions. This was noted both with hemocyanin and fibrinogen, but not with the agglutinin preparation (pale precipitate from serum) used. Boyden and deFalco (1943) reported a similar double zone phenomenon with *Homarus* serum titrated against anti-*Homarus*-hemocyanin. They pointed out that this is indicative of the presence of two kinds of antibodies in the antisera. However, this does not seem to be the entire explanation, since we find that absorption of a sample of antiserum with an amount of hemocyanin which corresponds to the lower of the two optima removes all antibody for the homologous antigen, as well as for fibrinogen and pale precipitate.

Two rabbits were also immunized with whole lobster-serum, each receiving a total of 5.5 ml. of serum in two courses of three injections each, with three weeks rest between courses. The antisera obtained one week after the last injection gave very good precipitation with the homologous antigen, optimal proportions (second optimum) being obtained with one volume of lobster-serum to approximately 16 volumes of antiserum. A sample was absorbed with purified hemocyanin and tested on whole serum, a concentrated solution of the pale (pH 5) precipitate, and a fibrinogen preparation. It failed to give precipitation with any of these antigens. This confirms the findings of Clark and Burnet (1942) and indicates that the other blood proteins have no active antigenic groups other than those present in the hemocyanin. Alternatively, the results might be explained on the basis of competition of antigens (see Sachs, 1929), such that the rabbit does not form antibodies against other antigens when one powerful antigen (the hemocyanin) is present in excess in the material (whole lobster-serum) used for immunization. However, in view of the analogous results obtained (Tyler and Metz, 1945) with antisera prepared against heteroagglutinin, and with antihemocyanin sera (above), the alternate explanation seems highly unlikely.

SUMMARY

1. Lobster-serum contains small amounts of other protein constituents besides hemocyanin.

2. The "class-specific" heteroagglutinins of lobster-serum are found to reside in a component that is obtained free of hemocyanin by isoelectric precipitation at pH 4.8 to 5.0.

3. Electrophoresis of this "pale precipitate" reveals the presence of two components, of which the more slowly migrating one bears the heteroagglutinating activity. The ten separate "class-specific" heteroagglutinins are thus evidently represented by a single electrophoretic component.

4. There is some indication that fibrinogen obtained from the lobster plasma may also act as heteroagglutinin.

5. Antibodies produced in rabbits against purified hemocyanin also react with the slow electrophoretic component (heteroagglutinin) of the pale precipitate and with fibrinogen. Absorption tests with antisera vs. whole lobster-serum fail to reveal the presence of any specific antigenic groups other than those of the hemocyanin. The other blood proteins are, then, evidently serologically equivalent to hemocyanin.

LITERATURE CITED

ALLISON, J. B., AND W. H. COLE, 1940. The nitrogen, copper and hemocyanin content of the sera of several arthropods. *Jour. Biol. Chem.*, **135**: 259–265.

CLARK, ELLEN AND F. M. BURNET, 1942. The application of the serological methods to the study of crustacea. *Austral. Jour. Exp. Biol. and Med. Sci.*, **20**: 89–95.

Boyden, A., and R. J. DeFalco, 1943. Report on the use of the photronreflectometer in serological comparisons. *Physiol. Zool.*, **16**: 229–241.

Longsworth, L. G., 1939. A modification of the Schlieren method for use in electrophoretic analysis. *Jour. Amer. Chem. Soc.*, **61**: 529–530.

Rawlinson, W. A., 1940. Crystalline haemocyanin: some physical and chemical constants. *Austral. Jour. Exp. Biol. and Med. Sci.*, **18**: 131–140.

Sachs, H., 1929. Antigene und Antikörper (c) die Konkurrenz der Antigene. *Handbuch der Norm. und Path. Physiol.*, **13**: 444–446.

Tiselius, A., 1937. A new apparatus for electrophoretic analysis of colloidal mixtures. *Trans. Faraday Soc.*, **33**: 524–531.

Tyler, A., and C. B. Metz, 1945. Natural heteroagglutinins in the serum of the spiny lobster, Panulirus interruptus. I. Taxonomic range of activity, electrophoretic and immunizing properties. *Jour. Exper. Zool.*, in press.

STUDIES ON MARINE BRYOZOA. I. AEVERRILLIA SETIGERA (HINCKS) 1887

. MARY DORA ROGICK

Marine Biological Laboratory and College of New Rochelle

TABLE OF CONTENTS

PAGE
Introduction 201
Distribution 201
Ecology 202
Description of species 203
Table I 205
Discussion 212
Summary 213
Literature cited 213
Explanation of Plate I 206
Explanation of Plate II 208

INTRODUCTION

During the summer of 1944 collections of *Aeverrillia setigera* were made at New Bedford and Woods Hole, Massachusetts. Perusal of literature pertaining to this species showed that a more complete account of this form would not be amiss. This article brings together all available distribution and anatomical data previously given for this form and adds to it some new distribution data, more complete illustrations than were heretofore available and a considerable amount of anatomical and some ecological data.

The writer wishes to acknowledge, with sincere appreciation, the kindness of Dr. Hannah Croasdale of Dartmouth College and of the Marine Biological Laboratory of Woods Hole, Mass., who collected the first specimens of *A. setigera* from New Bedford, Mass., and turned them over to the writer for study, and to Dr. Raymond C. Osburn of the University of Southern California who so kindly checked the specimens, confirming the identification and who offered many helpful suggestions.

DISTRIBUTION

The species *Buskia setigera* has been reported previously by the following authorities from the localities listed below:

Hincks, 1887 (pp. 121, 127-128; Pl. XII, Figs. 9-13), from the Gulf of Bengal, around the Mergui Archipelago.
Kirkpatrick, 1890a (pp. 603, 612), between Australia and New Guinea in the Torres Straits, 20 miles off Warrior Island.
Kirkpatrick, 1890b (p. 17), off Tizard Banks in the China Sea.
Thornely, 1905 (p. 128), from Ceylon.

Harmer, 1915 (pp. 87-88; Pl. 5, Figs. 8-10), from the Bay of Bima (India), Bay of Badjo, west coast of Flores (Malay Archipelago), Makassar, Borneo Bank, off Pulu Jedan, east coast of Aru Islands, and also in the following unnamed locations: Station 164, at 1°42′.5 S.; 130°47′.5 E.; Station 166, at 2°28′.5 S.; 131°3′.3 E.
Thornely, 1916, off Poshetra Head, Kattiawar, and Ceylon.
Hastings, 1927 (p. 351), at Menzaleh Lock and other stations at the Suez Canal.
Livingstone, 1927 (p. 67), from Queensland, Australia.
Hastings, 1932 (p. 407), from Penguin Channel and N. E. Low Island, Great Barrier Reef, Australia.
Osburn, 1933 (p. 64), from Porto Rico.
Marcus, 1937 (p. 143; Pl. 29, Fig. 76), from Bay of Santos, Brazil, South America.
Osburn, 1940 (p. 343), from Porto Rico.
Hutchins, 1945 (p. 539), Pine Orchard, Long Island Sound, Connecticut, U.S.A.

Additional discussion of the species occurs in the following articles:

Osburn and Veth, 1922; (p. 159).
Marcus, 1938; (p. 61).
Marcus, 1939; (pp. 168, 171).
Marcus, 1941; (pp. 74-77, 147; Pl. X, Fig. 45).

The above reports indicate that the species is distributed near several continents,—Africa (Suez Canal), Asia, Australia, South America, and North America, and also around several islands, including Porto Rico. The present article reports its occurrence around the State of Massachusetts, extending the northerly range of this species to 41°38′ N. Latitude.

Averrillia setigera was found in two Massachusetts localities. The first collection was made by Dr. Hannah Croasdale on July 29, 1944, at Black Rock in the Harbor of New Bedford, Mass. The next collections were made by the author on August 4, 13, and 14, 1944, at Stony Beach, Woods Hole, Mass. Further details of the nature of the collecting site and the associated biota will be given in the ECOLOGY section.

Ecology

The New Bedford Harbor specimens were collected by Dr. Croasdale at the time of low tide, from the littorine region around Black Rock, along with red algae, at a depth of less than 2 feet below the surface of the water. The Woods Hole specimens came from a large, partially submerged boulder located approximately 50 yards from shore. The sea bottom around the boulder is largely sand although there are some rocks a short distance away on each side of the boulder. The general locality is not subjected to strong wave action. The boulder is almost completely submerged at high tide but is about half exposed at low tide. Its sides are well covered with algae of various kinds as well as with a luxuriant fauna. The *A. setigera* colonies were collected at low tide, a foot or two below water level, by gathering likely looking *Chondrus* and *Ascophyllum* algae off the boulder.

The Woods Hole *A. setigera* specimens were found growing in close association with the following animal forms: *Folliculina, Vorticella, Sycon, Obelia, Sertularia,* other hydroids, *Bowerbankia gracilis, Bugula flabellata, Crisia eburnea,* *Hippothoa hyalina, Pedicellina cernua,* and *Stephanosella biaperta.* The *Aeverrillia* autozoids, and in some instances stolons, had a few *Folliculina, Vorticella,* or *Pedicellina,* growing on them. The *A. setigera* colonies grew on hydroid stems and on the same algal thalli (*Chondrus* and *Ascophyllum*) as *Bugula flabellata, Hippothoa hyalina, Crisia,* and the other Bryozoa.

Aeverrillia setigera has been collected from varying depths, from one or two feet below tide mark (present author) to much greater depths (other writers). Kirkpatrick found specimens at depths of 5½ and 27 fathoms; Thornely (1916), at 7 fathoms; Hastings (1932), at 8 to 15½ fathoms; Marcus (1937), at 17 meters; while Harmer found specimens at greater depths: 0 to 40 meters, 55 meters, 59 meters and 118 meters.

This Bryozoan grows on the following types of substratum: 1, on broken shells (Kirkpatrick, 1890a); 2, on stems of *Idia pristis* (Thornely, 1916); 3, on stems of hydroids and Bryozoa (Osburn, 1940); 4, on stems of *Nellia oculata* Busk (Hincks, 1887); 5, on hydroids and the following Bryozoa: *Bugula, Catenicella* and *Valkeria atlantica,* which were dredged from areas whose bottom consisted of such materials as mud, sand, hard coarse sand, coral, shells, and stones (Harmer, 1915); and 6, on hydroids like *Obelia* and algae like *Chondrus* and *Ascophyllum,* in close association with many other already mentioned animal forms (present paper).

Description of Species

The status of Bryozoa as an entire group is still an unsettled problem. It has been considered a Phylum, a Sub-phylum and a Class. Each category has its earnest and qualified supporters. With this in mind the following taxonomy of the *Aeverrillia* species, patterned after the work of Dr. Marcus, is given:

——— BRYOZOA Ehrenberg 1831
Class ECTOPROCTA Nitsche 1869
Order GYMNOLAEMATA Allman 1856
Sub-order CTENOSTOMATA Busk 1852
Group STOLONIFERA Ehlers 1876
Family Valkeriidae or Mimosellidae?
Genus *Aeverrillia* Marcus 1941
Species *setigera* Hincks 1887

The classification of *Aeverrillia setigera* has undergone a few changes since its original description by Hincks in 1887. Its generic names were *Buskia, Hippuraria,* and now *Aeverrillia.* The latter genus was erected in 1941 by Dr. Marcus in honor of A. E. Verrill.

The question regarding the family into which it should be placed is set forth by Marcus (1941, p. 147) thus: "*Aeverrillia* does not need a new family; the genus can be placed in the Valkeriidae or perhaps in the Mimosellidae as now enlarged by Bassler (1935, p. 8)." Earlier the species had been placed among the Triticellidae, the Buskiidae, and eventually into the Valkeriidae.

The colonies are delicate yellowish or very pale amber colored transparent traceries closely adherent to various living and non-living submerged objects. They are barely big enough to be seen with the unaided eye. They consist of paired individuals connected by slender stolons. The stolons and individuals are chitinized and firm-walled. The stolons especially have a well thickened wall.

Bryozoa exhibit polymorphism. The *Aeverrillia* colony consists of three types of structures or possibly individuals, namely stolons, peduncles, and autozoids.

In the colony there is a main or primary axis or stolon and lesser (secondary and sometimes tertiary) stolons (Fig. 7). The lesser stolons are more apparent in older colonies than in young ones.

These stolons, according to Dr. Marcus, are composed of kenozooecia. The long slender tubular kenozooecia of each stolon grow longitudinally and are attached end to end. Those of the secondary stolons have their origin at the sides of the primary stolon usually with a peduncle intervening between the primary and secondary stolons. The tertiaries have their origin at the sides of the secondaries, likewise usually with an intervening peduncle. Some stolons appear to arise directly from other stolons without an intervening peduncle (Fig. 8). Also, one of a pair of opposite stolons may arise from a stolon without the intervention of a peduncle while its partner may have a peduncle between it and its parent stolon (Fig. 8). Whether this barrenness of one stolon may be a temporary or a permanent condition is uncertain.

The primary and secondary stolons are at right angles, approximately, to each other but it is difficult to say the same about the tertiaries because the latter are sometimes twisted, gnarled, and not often found running in a straight line because of the limited area of the substratum on which the specimens grow. The secondary stolons usually originate in pairs, one stolon on each side of the primary stolon and directly opposite the other, growing away from each other.

The primary stolons, possibly because they are older, have thicker walls than the secondary stolons. The primaries are also somewhat straighter than the secondary and tertiary stolons but that again may be due to the limited substratum. Anastomoses occur occasionally, especially where there are tertiary and secondary stolons over a crowded or limited substratum. Hincks suggested the possibility of anastomosis of branches.

Primary stolons are very closely and entirely adherent to the substratum which in many cases proves to be a hydroid stem or *Chondrus* or *Ascophyllum* thallus. The primaries follow the stems or thalli in a fairly straight line for some distance. The secondaries and tertiaries must find what surface they can. Some of the lesser stolons look as if they are not necessarily attached along their entire length.

Generally the kenozooecia of the stolons are slightly enlarged distally at the point of origin of the lateral kenozooecia or peduncles. Transverse uniporous septa mark the proximal and distal limits of the kenozooecia along the stolons (Figs. 2 and 10). There are septa also at the points of origin of the lateral branches on the main stolons (Figs. 16 and 18). The region of the septum is sometimes referred to as the node and the stretch of stolon between two transverse septa, as the internode.

Stolon length is variable (Figs. 7 and 9, Table I). Some secondary stolons are short, some long. Some tertiary stolons are considerably longer than the primaries or than some of the secondaries. Stolon diameter is given in Table I.

The stolons under low power observation (75 × magnification) appear empty or tubular but under higher magnification (430 ×) a cellular lining membrane is evident within them.

TABLE I

Measurements of Massachusetts specimens of Aeverrillia setigera

Part	Number of readings	Maximum	Minimum	Average	Refer to Figs.
A. Length or height of furled setigerous collar	17	0.602 mm.	0.440 mm.	0.531 mm.	6, 17
B. Diameter at distal end of unfurled setigerous collar	5	0.537 mm.	0.370 mm.	0.440 mm.	6
C. Diameter at the basal, proximal end of the setigerous collar	6	0.110 mm.	0.059 mm.	0.083 mm.	6, 17
D. Length of orificial spine	20	0.259 mm.	0.141 mm.	0.204 mm.	6
E. Diameter of extruded vestibular membrane	1			0.111 mm.	6
F. Length of extruded vestibular membrane	1			0.321 mm.	6
G. Length of tentacular sheath	1			0.237 mm.	6
H. Diameter of tentacular sheath	2	0.096 mm.	0.074 mm.	0.085 mm.	6, 11
I. Autozoid width at widest part	7	0.212 mm.	0.170 mm.	0.185 mm.	8
J. Autozoid length from base of zoid to base of orificial spines	18	0.592 mm.	0.481 mm.	0.552 mm.	8
K. Stolon diameter, at the normal thickness, not the swollen area of the stolon	24	0.049 mm.	0.015 mm.	0.027 mm.	8
La. Length of shorter lateral surface of the clasping processes	23	0.179 mm.	0.043 mm.	0.110 mm.	7
Lb. Length of longer lateral surface of the clasping processes	23	0.182 mm.	0.077 mm.	0.119 mm.	7
M. Width of stolon at most swollen part, near node	18	0.051 mm.	0.034 mm.	0.040 mm.	8
N. Length of peduncle	19	0.170 mm.	0.068 mm.	0.114 mm.	18
O. Diameter of peduncle	19	0.071 mm.	0.039 mm.	0.058 mm.	8
P. Length of internode	24	1.013 mm.	0.294 mm.	0.658 mm.	9
S. Number of tentacles	6	8	8	8	6, 13, 14
T. Number of setae in setigerous collar	3	19	16	17	6

PLATE I

All figures except Figures 2 and 6 have been drawn with the aid of a camera lucida. All are of *Aeverrillia setigera.*

Figure 1. A chitinized sconce of the proventriculus, seen from the lumen side. Note the converging rows of teeth. Drawn to the same scale as Figure 10. This and Figures 3, 4, and 5 are from gizzard remains found in empty, degenerated autozooecia.

Figure 2. Detail of the uniporous septum which occurs along the stolons.

Figure 3. Latero-basal view, from the concave side of the chitinized gizzard sconce. All the softer parts of the gizzard have disintegrated, leaving only the hardened plate or sconce. Drawn to the same scale as Figure 10.

Figure 4. Side view of a somewhat flattened chitinized gizzard sconce. Drawn to the same scale as Figure 10.

Figure 5. Side view of a chitinized gizzard sconce of the more usual shape. Some of the teeth are darker than others. Drawn to the same scale as Figure 10.

Figure 6. A diagram showing several things: the relation between the open or unfurled setigerous collar, the eight tentacles, three of the four zooecial spines around the orifice and the lettered areas A through G along which measurements for Table I have been made. The same letters are found listed in the first column of the Table.

Line A stands for the length or height of the setigerous collar. It was measured only when furled or very slightly unfurled.

Line B represents the diameter of an unfurled setigerous collar, at its distal, expanded end.

Line C represents the diameter at the basal or proximal end of the setigerous collar.

Line D represents the length of the orificial spine.

Line E represents the diameter of the vestibular membrane and the area it encloses.

Line F represents the length of the vestibular membrane.

Line G represents the length of the tentacular sheath or the distance between the lophophore and the base of the setigerous collar, C——C.

Figure 7. Part of an old zoarium or colony showing the growth habit, anastomosis of branches (AN), primary stolon (P.ST.) and secondary stolon (S.ST.). All the autozooecia are empty of polypides. One (Z) has the setigerous collar in place yet but has no polypide. Some of the zooecia have two or three acuminate processes (B.P.). Measurements of these acuminate processes were made along two surfaces, the shorter (La) and the longer (Lb). These figures are to be found in Table I. The "membranous" area mentioned by Hincks is not very plain on most specimens. However, there is a hint of it in the second and fourth zooecia from the top. Drawn from freshly collected material on Aug. 14, 1944, and to the scale shown at its base.

Figure 8. An autozooecium attached to a very long secondary stolon. The location of certain measurements mentioned in Table I is indicated on the drawing.

Line I represents the diameter of the autozoid.

Line J represents the length or height of the autozoid exclusive of spines.

Line K represents the diameter of the stolon along most of its length and not at the swollen areas.

Line M represents the diameter of the stolon at the slightly swollen node region.

Line O represents the height or thickness of the peduncle.

The faintly curving line along the autozooecium suggests the location of the "membranous" area. The scale below belongs with this sketch.

Figure 9. Part of a colony showing four autozoids growing quite regularly in pairs, on peduncles at opposite sides of the primary stolon. Two are empty, the third has a setigerous collar and the fourth has a living polypide within. A darker gizzard is evident within the last. The scale directly below belongs to this colony.

Figure 10. The section of the stolon showing a septum and the swollen part represented by M in Figure 8. This is the region of the node. Drawn to the scale directly below. Figures 1, 3, 4, 5, and 11 also are drawn to this scale.

Figure 11. Part of an autozoid showing the basal region of the setigerous collar through which is lightly indicated the tentacle-bearing lophophore and tentacular sheath. At the base of the setigerous collar are placed two small letters H which represent the width of the tentacular sheath. The measurements are found in Table I. Below the setigerous collar is the vestibular membrane through which are visible muscle fibers. Drawn to the same scale as Figure 10.

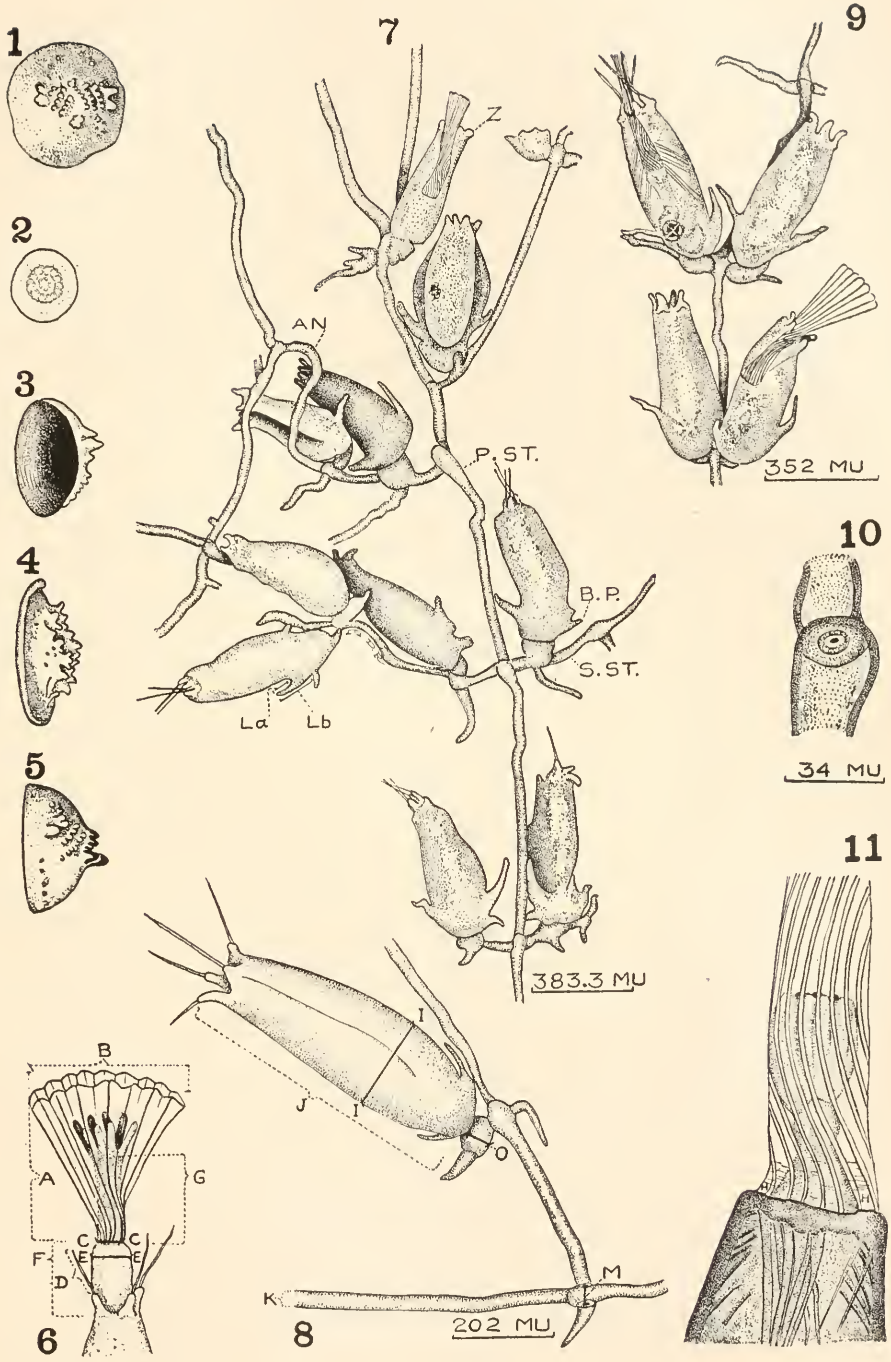

1
2
3
4
5
7
Z
AN
P. ST.
B. P.
S. ST.
La
Lb
383.3 MU
9
352 MU
10
34 MU
11
H
H
B
A
G
C
C
E
E
F
D
6
I
I
J
O
M
K
202 MU
8

PLATE I

The second type of structure or possibly individual (?) in *A. setigera* is the peduncle, so designated by Marcus (1937, p. 142). This is a short much swollen segment generally placed between stolons which are at right angles to each other and found at the base of the autozoids (Figs. 8 and 16). It originates from a stolon and gives rise to a stolon and an autozoid. It is cut off from the stolons and autozoid by a uniporous septum. A peduncle is more swollen and of shorter length than the stolon kenozooecium and has a lining membrane. In one instance there appeared a few transverse fibers inside a peduncle.

The third type of individual in an *A. setigera* colony is the autozoid. It arises from the peduncle. The autozoids are just big enough to see with the unaided eye. Harmer (1915, p. 87) gave their length as 0.48–0.55 mm. and Osburn (1940, p. 343) as 0.50–0.60 mm. Their width was given as 0.18 mm. (Harmer, 1915 and Osburn, 1940). Measurements of the Massachusetts specimens are given in Table I.

The autozoids occur in pairs bilaterally placed with respect to the primary stolon (Fig. 9). Where secondary stolons are well developed the autozoids occur in the same manner along the secondary stolon. Occasionally one of the paired autozoids is missing but a stub of its peduncle or a stolon may be present in its place (Fig. 8). These paired autozoids are not truly parallel but converge slightly basally as shown

---

PLATE II

All figures are drawn with the aid of a camera lucida and are of *Aeverrillia setigera*.

FIGURE 12. A part of the unfurled setigerous collar, showing the delicate transparent membrane which folds like a fan. Its stiff supporting ribs or setae are transparent also. Drawn to the scale at left.

FIGURE 13. An autozoid in which a very young polypide is growing. A characteristic setigerous collar is not yet present although its Anlage (SC) is visible. Eight tentacles can be counted. The digestive tract is small. A gizzard or proventriculus is present in it. Drawn from living material on August 13, 1944, to the scale shown directly below.

FIGURE 14. Another young autozoid but slightly older than that of the preceding figure. Drawn to the same scale.

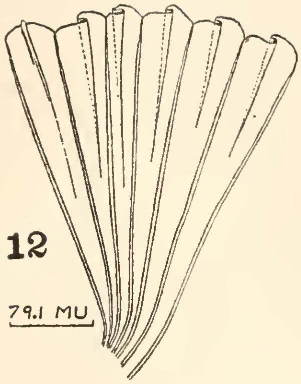
FIGURE 15. View of a mature autozoid showing an almost completely retracted polypide, a very long folded setigerous collar partially withdrawn, the U-shaped digestive tract twisted around in the lower half of the zooecium. The gizzard (GZ) is oriented in such a manner that one is looking along its vertical axis. Some of the body wall and polypide musculature is shown, particularly the circularly arranged parietal muscles (PM). The acuminate process is barely visible. Drawn to the same scale as Figure 12.

FIGURE 16. A partly retracted autozoid. The tentacle tips are just barely visible in the dark mass at the base of the spine-bearing processes. The somewhat indistinctly depicted digestive tract is in the basal part of the zooecium. Only a part of the autozoid at left is shown. The scale directly above the setigerous collar applies to this figure.

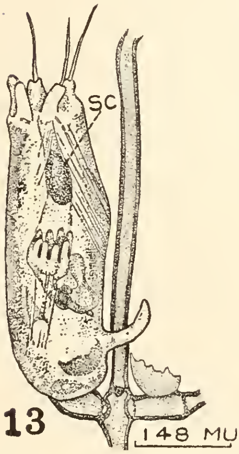
FIGURE 17. A folded setigerous collar showing typical twisting of the supporting ribs or setae. The transparent membrane is faintly indicated at the distal end. Drawn to the scale directly below.

FIGURE 18. A young autozoid is shown at left. Only a part of the right one is included. The young polypide has eight tentacles and a U-shaped digestive tract. The setigerous collar is not visible but its Anlage (SC) is present. The vestibule (V) and the parieto-vaginal muscles (PVM) are plain. Line N represents the length of the peduncle which bears the autozoid. Measurements of it are given in Table I. Drawn to the same scale as Figure 13, on Aug. 13, 1944, from fresh material.

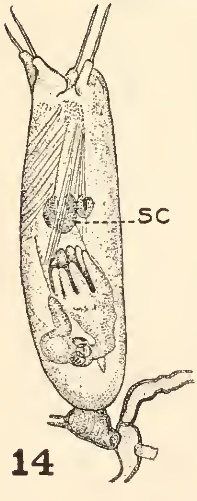
FIGURE 19. Three of the four chitinized gizzard sconces. The teeth are darker than the rest of the disc in this particular case. Muscle fibers encircle the cluster of four sconces and are here indicated by horizontal or parallel lines. Drawn to the scale at left.



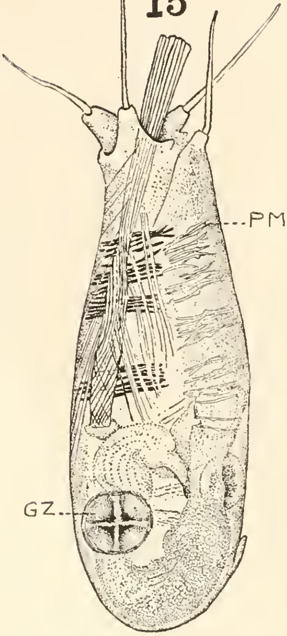
12



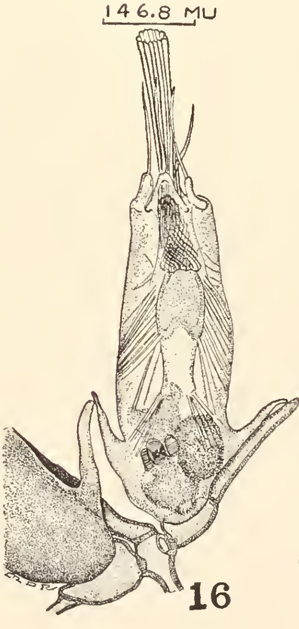
13



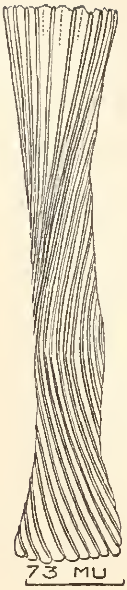
14



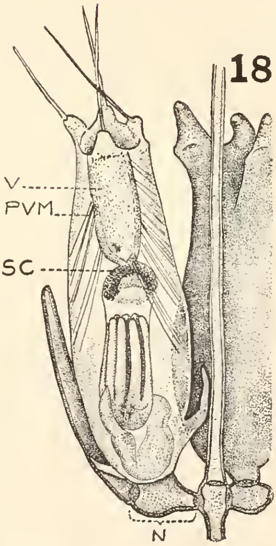
15



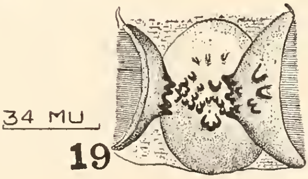
16



17



18



19

PLATE II

in Figure 9. They are not upright but are recumbent at an angle close to the substratum. The basal part rests directly on the substratum, or is very close to it, while the distal part is free. The autozoids are somewhat elongate ovate with the broad end attached. The side nearest the substratum and the inter-autozoidal stolon is slightly flatter than the opposite side. At its point of origin the autozoid may be globose as in Figure 14 or slightly "stemmed" as in the right-hand individual of Figure 18.

The lower half of the autozoid is swollen slightly. From it arise from one to four, usually two, acuminate clasping processes (Figs. 7 and 16), which were called "tubular adherent processes" by Hincks (1887, p. 128) and "spines" by Harmer (1915, p. 87). They are placed obliquely on the zoid. They may touch the stolons or the neighboring autozoid or else cling to the substratum without touching either the adjacent autozoid or the stolon. Colonies in place on hydroids show some of these clasping processes curling around the hydroid stems, closely adherent. These clasping processes are hollow and not separated by any sort of septum from the rest of the zoid.

Hincks (1887, p. 127) described a large aperture closed by a membranous wall on the greater part of the ventral side of the autozoid. It is difficult to see in the Massachusetts specimens although indications of it are present in Figures 7 and 8. In Figure 7, it is evident on the second and fourth autozoids from the top. Moreover, it appears chitinized rather than membranous.

The distal tapering end of the autozoid has four spine-bearing processes (basal segments or flaps). Occasionally more than four flaps may occur. Harmer (1915, p. 88) reported a specimen with eight. This condition however is very infrequent. These flaps are arranged around the zooecial orifice through which the setigerous collar may be protruded.

The position of these distal triangular flaps is not rigidly, immovably fixed. The line of bending is at the base of the triangle. Sometimes the flaps may be flexed inward so that their spines may cross each other above the orifice as in the top left-hand zoid of Figure 9, or in Figure 16. This is the usual position when the setigerous collar is withdrawn into the autozoid. When the setigerous collar is out the flaps are bent outward as in Figure 6. This is the condition also in many empty zooecia. Whether there are any muscle fibers controlling the movement of these flaps was impossible to determine from the material at hand.

The flaps are more heavily chitinized than the surrounding zooecial wall. The difference is quite noticeable.

The apex of a triangular flap is rounded in all views. A sharply tapering, slightly irregular orificial spine is set shallowly into this rounded area. The spine is hollow, but so far as it is possible to determine its cavity is not continuous with the cavity of the flap but is cut off by a septum. In Porto Rican specimens the spines measured 0.20–0.30 mm. (Osburn, 1940, p. 343). Measurements of Massachusetts specimens are given in Table I.

The setigerous collar is long and very slender when furled. Harmer (1915, p. 88) gives its length as 0.46 mm. and its breadth at the distal end as 0.130 mm. This last figure is undoubtedly of a partly furled individual. The dimensions of the Massachusetts specimens are included in Table I.

The setigerous collar can be protruded clear out of the autozoid (Fig. 6). On the other hand, it also can be completely withdrawn into the autozoid. In fact it can be pulled in so far that its uppermost or distal tip is halfway down inside the zoid. There are muscular fibers attached to its base (Fig. 11). When it is completely withdrawn the tentacles are below it. When it is protruded and expanded the tentacles are within its circle of setae (Fig. 6).

Hincks (Pl. XII, Fig. 13), Harmer (Pl. V, Fig. 9), Marcus (1937, Pl. XXIX, Fig. 76) and the present writer (Figs. 6, 11, 15, and 17) have pictured the peculiar spiral twisting of the setae of the collar. The setae reinforce a delicate, colorless, transparent membrane which folds neatly like a fan along scarcely discernible creases between adjacent setae, when the collar is being withdrawn (Figs. 6, 12, and 17). The setae or ribs supporting the collar are extremely regular in diameter from base almost to the very tip.

The collar is often found in excellent condition even when all the zoid contents except the zooecial wall have disintegrated.

In young zoids as represented in Figures 13, 14, and 18 the setigerous collar is not yet completed but is represented by a mass of germinative tissue, SC, which temporarily forms a flexible canopy above the tentacles, at the bottom of the vestibule.

The vestibule is the cavity down which the setigerous collar travels when being withdrawn. Its wall is formed by a soft vestibular membrane, to which are attached a number of fibers which constitute the parieto-vaginal muscles. The vestibular membrane is shown withdrawn or introverted in Figure 18 and extruded in Figure 6.

The circular lophophore bears eight tentacles (Figs. 6, 13, and 14). This number is in agreement with the statements of Harmer and Marcus.

The tentacles, when retracted, are pulled into the introverted tentacular sheath in a manner characteristic of the Bryozoa (please compare Figs. 6 and 18).

They surround the entrance to the digestive system which is a U-shaped tract consisting of pharynx, esophagus, proventriculus, stomach and intestine. The most interesting features about the tract are the great length of the esophagus and the presence of a muscular and chitinized proventriculus or gizzard between the stomach and esophagus.

The proventriculi of various species of *Buskia* or *Aeverrillia* are illustrated in papers by Osburn and Veth (1922, Plate I) and Marcus (1941, Plate X, Figs. 44B and 45). Marcus figures the gizzard of both *A. armata* and *A. setigera*. However, the proventriculus of the Massachusetts specimens of *A. setigera* resembles that of his *A. armata* as much as it does that of his *A. setigera*.

The proventriculus of the Massachusetts *A. setigera* is a compact, rounded organ consisting of four conical chitinous sconces capping the internal epithelium. A wide band of circular muscle fibers surrounds these four sconces (Fig. 19). An end view of the proventriculus showing the relation of the four sconces to each other is pictured clearly in Figure 15 and suggested in Figures 9 and 18. A side view, showing the relation of the circular musculature to the sconces and the relative position of the proventriculus in the polypide, is depicted in Figures 13, 14, and 16. A detailed picture of the arrangement of the chitinous and sometimes brown-colored denticles on the sconces appears in Figures 1, 3, 4, 5, and 19. The denticles seem to have a definite arrangement in several roughly V-shaped rows. They are of various sizes. Their color ranges from pale yellow to brown. The shape of each sconce at the base ranges from a broad ellipse to a circle. In side view the sconce may appear globose, conical, or even slightly flattened, except for the projecting teeth. Careful inspection of an old or empty colony may occasionally reveal sconces of degenerated polypides still within the otherwise empty zooecia. Because the sconces are usually transparent, pale yellow, and small it is easy to overlook them. In degenerating polypides the gizzard can usually be distinguished as the central part of a dark mass of degenerating material.

The relations of the stomach and intestine to the gizzard and to the lophophore can be seen in Figures 13, 14, and 18. In these three instances the digestive tract is empty. In a mature feeding individual the digestive tract is considerably longer, as a study of Figure 15 will show. The intestine opens outside the circle of tentacles—a characteristic of the Ectoprocta.

The musculature of the lower half of the autozoid was difficult to study partly for lack of sufficient living material and partly because in a mature zoid the digestive tract occupies so much of the interior. However Figure 13 does show a suggestion of a band of retractor muscle fibers attached to the base of the tentaculer crown or the upper part of the digestive tract.

The other major muscles attaching to the body wall are the horizontally or circularly arranged parietal muscles. Harmer (1915 p. 88) states that three groups of parietal muscles are visible in his specimens. In the Massachusetts specimens it appears as if there are four groups (Fig. 15).

In a few near-empty zooecia, from which the musculature, tentacles, setigerous collar, and digestive tract were missing but which had a brown body (a mass of dedifferentiating or degenerating tissue) in the upper half of the zooecium, was noticed a rather peculiar globular membranous sac attached to the base of the interior of the autozoid, in the vicinity of the septum which separates the autozoid from the peduncle. This globose mass was hollow. Its wall was soft membranous, and turgid. It is not figured here. Its appearance and position suggest one of two possibilities: 1, it may be a regenerating mass which would give rise to a new polypide within the old zooecium; or 2, it may represent the remains of a degenerating polypide, exclusive of the brown body which was already evident in the upper half of the zooecium. In the fresh water Bryozoa, when polypides of a colony degenerate, sometimes the wall of the colony forms a hollow membranous sac which may either degenerate completely or give rise to a new colony (Rogick, 1938; p. 197).

In studying any form, measurements are extremely helpful. Therefore, as complete a set of measurements of *A. setigera* as was possible was made and is arranged in Table I. The letters and parts A to P are clearly indicated in the drawings of Plates I or II.

DISCUSSION

Aeverrillia setigera seems very widely distributed circumtropically. It has been reported previously from such widely scattered localities as north and east of Australia, China Sea, Gulf of Bengal, Malay Archipelago, Suez Canal, Porto Rico, Brazil's Bay of Santos, etc., whose latitudes range from approximately 24° S to 31° N. The present report extends its range to 41°38′ N. Latitude. A recent report (Hutchins, 1945; from Long Island Sound) cites its occurrence slightly south of the present paper. In spite of this extensive range the number of reports on the occurrence of this species have not been too numerous: Harmer, Hastings, Hincks, Hutchins, Kirkpatrick, Livingstone, Marcus, Osburn, Thornely, and the present writer.

The Massachusetts specimens agree essentially in measurements and appearance with those found in more southerly waters (Gulf of Bengal, South America, and Porto Rico) by previous workers.

Because of their small size and inconspicuous appearance they are easily overlooked when collecting. Very little is known of their behavior, embryology, life history, and physiology. A study should be made of these as well as of colony degeneration, regeneration, rate of growth, development of the proventriculus and setigerous collar, the location and development of the reproductive system, and the nature of the larva. All the work done so far on this form has been of taxonomic nature. The present paper has added a more complete account of the anatomy, included measurements of a number of parts hitherto unmeasured and added a more complete series of diagrams than have existed previously for this species.

SUMMARY

1. *Aeverrillia setigera* was found at Woods Hole and at New Bedford, Mass. This extends its northerly range to 41°38′ N. Latitude.

2. The Massachusetts specimens agree closely in appearance and measurements with specimens from more southerly waters of the Gulf of Bengal, Malay Archipelago, South America, and Porto Rico.

3. Measurements of many structures or parts not measured by other workers are here included.

4. The species has been more fully illustrated.

5. The species did not seem to be abundant in the localities from which it has just been reported.

LITERATURE CITED

BASSLER, R. S., 1935. Fossilium Catalogus. I. Animalia, pars **67**: Bryozoa. *'s-Gravenhage.* p. 1–229.

HARMER, S. F., 1915. The Polyzoa of the Siboga Expedition, Part 1. The Entoprocta, Ctenostomata and Cyclostomata. *Siboga-Expeditie, Mongr. 28a, Livr.* **75**: 1–180; Pl. 1–12.

HASTINGS, A. B., 1927. Report on the Polyzoa of the Suez Canal. *Trans. Zool. Soc. London,* **22** (pt. 3, no. 8): 331–354.

HASTINGS, A. B., 1932. The Polyzoa, with a note on an associated hydroid. *Great Barrier Reef Exped. 1928–29, Sci. Reports,* **4** (12): 399–458, Pl. 1. *Brit. Mus. Nat. Hist.*

HINCKS, T., 1887. On the Polyzoa and Hydroida of the Mergui Archipelago collected . . . by Dr. J. Anderson. *Jour. Linn. Soc. Zool.,* **21**: 121–135; Pl. 12.

HUTCHINS, L. W., 1945. An annotated check-list of the salt-water Bryozoa of Long Island Sound. *Trans. Conn. Acad. Arts and Sci.,* **36**: 533–551.

KIRKPATRICK, R., 1890a. Hydroida and Polyzoa. Reports on the Collection made in Torres Straits by Prof. A. C. Haddon 1888–1889. *Sci. Proc. R. Dublin Soc., n.s.,* **6**: 603–625; Pl. 14–17.

KIRKPATRICK, R., 1890b. Report upon the Hydrozoa and Polyzoa collected by P. W. Bassett-Smith . . . during the survey of the Tizard and Macclesfield Banks in the China Sea, by H.M.S. "Rambler." *Ann. Mag. Nat. Hist., ser. 6,* **5**: 11–24; Pl. 3–5.

LIVINGSTONE, A., 1927. Studies on Australian Bryozoa, No. 5. A checklist of the marine Bryozoa of Queensland. *Rec. Austral. Mus.,* **16** (1): 50–69.

MARCUS, E., 1937. Bryozoarios marinhos brasileiros, I. *Univ. São Paulo, Bol. Faculd. Filos., Ciênc. e Letr., I. Zool.,* **1**: 3–224; Pl. 1–29.

Marcus, E., 1938. Bryozoarios marinhos brasileiros, II. *Univ. São Paulo, Bol. Faculd. Filos., Ciênc. e Letr., IV. Zool.,* **2**: 1–196; Pl. 1–29.

Marcus, E., 1939. Briozoarios marinhos Brasileiros. III. *Univ. São Paulo, Bol. Faculd. Filos., Ciênc. e Letr., XIII. Zool.,* **3**: 111–354; Pl. 5–31.

Marcus, E., 1941. Sôbre os Briozoa do Brasil. *Univ. São Paulo, Bol. Faculd. Filos., Ciênc. e Letr., XXII. Zool.,* **5**: 3–208; Pl. 1–18.

Osburn, R. C., 1933. Bryozoa of the Mount Desert Region. *Biol. Surv. Mt. Desert Region,* Part **5**: 291–385; Pl. 1–15.

Osburn, R. C., 1940. Bryozoa of Porto Rico with a résumé of the West Indian Bryozoan Fauna. *Sci. Surv. Porto Rico and the Virgin Islands,* **16** (3): 321–486; Pl. 1–9. *N. Y. Acad. Sci.*

Osburn, R. C., and R. M. Veth, 1922. A new type of Bryozoan gizzard, with remarks on the Genus *Buskia. Ohio Jour. Sci.,* **22** (6): 158–163.

Rogick, M. D., 1938. Studies on Fresh Water Bryozoa. VII. On the viability of dried statoblasts of *Lophopodella carteri* var. *typica. Trans. Amer. Micr. Soc.,* **57** (2): 178–199.

Thornely, L. R., 1905. Report on the Polyzoa . . . at Ceylon, in Herdman's *Rept. Ceylon Pearl Oyster Fish.,* vol. **4**, Supplement. Report, 26: 107–130.

Thornely, L. R., 1916. Report on the Polyzoa collected at Okhamandal in Kattiawar in 1905–1906. Hornell, *Rept. Gov. Baroda Mar. Zool. Okhamandal,* Part **2**: 157–165.

STUDIES ON FRESH-WATER BRYOZOA. XVI. FREDERICELLA AUSTRALIENSIS VAR. BROWNI, N. VAR.

MARY DORA ROGICK

Marine Biological Laboratory and College of New Rochelle

TABLE OF CONTENTS

PAGE
Introduction 215
Fredericella australiensis (emend.)
- Description 216
- Discussion 217
  - Growth habit 217
  - Dissepiments or septa 217
  - Keel 217
  - Zooecial tube 217
  - Ectocyst 218
  - Polypide 218
  - Tentacular crown 218
  - Sessoblasts 224
- Distribution 225
- Key to varieties 226
- Table I 219

Fredericella australiensis var. *browni*
- Description and discussion 226
- Table II 225

Summary 227
Literature cited 228
Plate I, Explanation 220
Plate II, Explanation 222

INTRODUCTION

This study deals with a *Fredericella, F. australiensis* Goddard 1909, which was reduced to variety rank and to which were added two other varieties, one of them new. The new variety is here named *F. australiensis* var. *browni,* in honor of Dr. Claudeous J. D. Brown of the Michigan Department of Conservation, Ann Arbor, Michigan, who most generously turned over the material to the author for further study.

The specimens were collected in fair abundance on August 3, 1942, from rocks in an alkali pond about three miles northeast of Church Butte, Uinta County, Wyoming, U.S.A., by Dr. Henry van der Schalie of the University of Michigan, at Ann Arbor.

The writer wishes to express her deep appreciation to both Dr. van der Schalie and Dr. Brown for the opportunity to examine the specimens and to make the present study.

Observations were made on preserved material which was dissected and on preserved material which had to be imbedded and sectioned. No living specimens were available. Dissection and sectioning were necessary to determine tentacle number, diameter of various parts, and internal structure since the zooecial wall was too opaque to permit ready observation of internal structures.

It was necessary to create a new variety, var. *browni*, for the Wyoming form because it resembled very closely in some respects and differed somewhat in other respects from two other forms known heretofore as *Fredericella sultana* subsp. *transcaucasica* Abricossoff 1927 and *Fredericella australiensis* Goddard 1909.

It was necessary to reduce the original *F. australiensis* of Goddard to variety rank, making it *F. australiensis* var. *australiensis* and to add to it two other varieties because the three forms so closely resembled each other and differed noticeably from the long established species of *Fredericella sultana*. Consequently, the former *F. australiensis* Goddard and the *F. sultana* subsp. *transcaucasica* Abricossoff become varieties under the emended *F. australiensis*, namely, *F. australiensis* var. *australiensis* and *F. australiensis* var. *transcaucasica*. The finding of the Wyoming specimens adds a third variety, *browni* to this emended species.

FREDERICELLA AUSTRALIENSIS, EMENDED

Description

The colony is attached along the bases of a number of zooecia whose tips become erect at the distal end and eventually give rise to upright branches which usually do not fuse into a solid mass but which form rather openly branched tufts (Fig. 4). Branching is antler-like or very roughly dichotomous. Septa or dissepiments are absent. Zooecial tubes are slightly wider than those of *F. sultana*. The degree of incrustation of the ectocyst varies from almost none in var. *transcaucasica* to a considerable amount in var. *browni* and var. *australiensis*. Floatoblasts are absent. Sessoblasts are rounded or very broadly elliptical, not reniform or very elongate as those of *F. sultana*. They are shorter and broader than those of *F. sultana*. More exact data or measurements will be given in the "Discussion" section. The terms sessoblasts and floatoblasts have been defined in the author's Study XIV. The *F. australiensis* polypides are shorter and stubbier than those of *F. sultana* and are restricted to the zooecial tips whereas those of the latter species are longer and extended further down into the zooecial tubes. The tentacle number is larger in *F. australiensis* than in *F. sultana*. The former has approximately 24 to 30 tentacles while the latter has about 17 to 24 tentacles. The lophophore is decidedly elliptical in var. *australiensis*. In the other two varieties it is uncertain whether the lophophore is nearly circular or definitely elliptical. Living specimens are necessary to determine this point. However, the lophophore is not horseshoe-shaped, except only in the retracted condition. An epistome is present.

Fredericella australienisis is characterized by the rounded, broadly elliptical shape of the sessoblasts, the larger number of tentacles and greater zooecial tube diameter, all admittedly somewhat variable characters but unfortunately almost the only ones, barring nature of colony growth and degree of incrustation which in themselves are variable, on which one can make a distinction in this genus.

Discussion

Growth habit

Fredericella australiensis and *F. sultana* have a similar growth habit and colonial appearance. The mode of branching is similar. Zoids are adherent for a distance then give off upright branches. Branching is antler-like or very roughly dichotomous in both.

Dissepiments or septa

Allman (1856, p. 112) says of *F. sultana,* "At the origin of the branches there is frequently found a more or less perfect septum." His Plate IX, Figure 3, shows an imperfect or partial septum, i.e., a septum with a hole in it. This chitinous septum is located at the commencement of a branch. Kraepelin (1887) calls the dissepiments rudimentary. In *F. australiensis* there seem to be no septa at the start of the branches. Goddard (1909, p. 490) finds none in var. *australiensis.* Abricossoff (1927b, p. 88) shows none in his Figure 2 of *transcaucasica,* and there appear to be none in var. *browni* (present study).

Keel

There seems to be relatively little difference between *F. sultana* and *F. australiensis* in this character. The zooecial tubes are cylindrical or nearly so in younger *F. sultana* zooecia and keeled in older specimens, so there occur specimens with and without a keel. This is true also of *F. australiensis*—some individuals may have and others may lack a keel.

Zooecial tube

The two species differ very slightly in the shape of the zooecial tubes, when viewed in cross section. The *F. sultana* tubes vary in cross section from cylindrical in unkeeled specimens to somewhat pear-shaped in keeled ones. In *F. australiensis* the tube cross section ranges from an ellipse (in var. *browni,* Figs. 1 and 10) to a rough triangle (var. *australiensis*).

There is a greater difference between the two species in width of zooecial tubes. Those of *F. sultana* are more slender. The diameter of *F. sultana* zooecial tubes of New Rochelle and Lake Erie specimens as given in Study IX (Rogick, 1940, p. 195) ranged from 0.16 to 0.35 mm. and averaged 0.24 mm. for 44 readings. Abricossoff (1927b, p. 91) said that in the U.S.S.R. *Fredericella sultana* the zooecial tube was not more than 0.4 mm. wide. He placed that as the upper limit but did not give the minimum nor average measurements for the point in question. The zooecial tubes of *F. australiensis* are greater in diameter than those of *F. sultana.* Abricossoff (1927b, p. 91) gives the average diameter in *transcaucasica* as 0.5 mm. while the present writer gives a range of 0.259 to 0.576 mm. or an average of 0.391 mm. for the most typical region of a var. *browni* zooecial tube. Thus it would seem that as regards this particular character, var. *browni* is somewhat closer to *F. sultana* than is var. *transcaucasica.*

Ectocyst

There is little difference in appearance between the two species so far as chitinized ectocyst is concerned. In *F. sultana* the degree of incrustation of the ectocyst may vary to such an extent that the zooecial tubes may be translucent to opaque, generally favoring the latter. Debris, stone particles and even algae may attach to it. In *F. australiensis* the degree of incrustation varies also from extremely little in var. *transcaucasica* to the usual "opaque," reasonably well incrusted amount in the other two varieties. Sand grains and debris form part of the incrustation. The color of the ectocyst varies from tan to light brown, in *F. australiensis.*

Polypide

Kraepelin (1887, p..99) says that polypides of *F. sultana* are very long. Allman (1856, Pl. IX, Fig. 7) shows such a specimen. In samples observed by various workers, including the present one, the polypides of this species seemed long and slender. On the other hand, in *F. australiensis,* the polypides appear distinctly shorter and stubbier, and are restricted to the zooecial tips (see Goddard, 1909, Fig. 12). Since no digestive tract measurements exist for *F. sultana* it is necessary to judge the relative length of its tract by studying Allman's and other workers' drawings. These measurements would vary with the age and condition of nourishment of the polypides.

Tentacular crown

In *F. sultana* the tentacles are long and slender but no measurements exist for them so far as can be determined. In *F. australiensis* the tentacles are generally shorter and stubbier with the possible exception of var. *australiensis.* In the latter variety they measure about one mm. in length and 0.01 mm. in diameter. In var. *browni* the tentacles are shorter and thicker. Unfortunately not too many were in a position to be measured accurately so that one had to depend on the general appearance of those dissected out of the colonies and on a few which were sectioned in the proper plane. These ranged from 0.383 to 0.514 mm. in length and from 0.019 to 0.029 mm. in width (Table II). This is shorter and wider than in var. *australiensis.* No measurements are available for var. *transcaucasica* tentacles. One has to judge them from Abricossoff's (1927b, p. 88, Fig. 2) figure in which they appear shorter and stubbier than tentacles of his *F. sultana* (*ibid.,* Fig. 1).

The number of tentacles does not seem to vary as much in *Fredericella* individuals as it does in those of *Plumatella* and *Hyalinella.* In *Hyalinella punctata,* the author (1945, Study XV. p. 69) found that the ancestrula or first polypide of a colony could be distinguished from successive polypides on the basis of the number of tentacles. It had about 10± less than successive polypides did. Whether the same general principle holds for *Fredericella* and other fresh-water forms could easily enough be determined by germinating statoblasts of the various forms and keeping accurate counts of the number of tentacles developed in each zoid.

The tentacle number of the two species of *Fredericella* is different. In *F. sultana* it ranges from 17 to 24, with 20 to 22 being the most common number. In *F. australiensis* the number ranges from 24 to 30.

TABLE I

Comparison of the three varieties of Fredericella australiensis and including Borg's African specimens

Part or structure	var. *australiensis*	var. *browni*	var. *transcaucasica*	Borg's African specimens
A. Lophophore x-section			no data given	no data given
1. expanded?	about 0.38×0.23 mm.			
2. retracted		0.182×0.133 mm. average		
B. Sessoblast length and width	no data given	0.382 × 0.316 mm. average	0.470×0.315 mm. average	Type A sessoblasts, 0.37–0.43 mm. long by 0.22–0.27 mm. wide Type B sessoblasts, 0.33–0.40 mm. long by 0.30–0.35 mm. wide
C. Zooecial tube diameter	no data given	0.391 mm. average	0.5 mm. average	Creeping part of tube, 0.33–0.45 mm. Erect part of tube, 0.24–0.33 mm.
D. Tentacle number	28–30	24–28	no data given	24–28; usually 26–27
E. Ectocyst appearance	chitinous, brown, incrusted	chitinous, well incrusted, tan, quite opaque	chitinous, thick, light brown, transparent, very little incrusted	chitinous, considerably incrusted with sand grains
F. Zooecial tube in x-section	roughly triangular	elliptical	no data given	Some strongly keeled. Triangular in attached parts of colony and rounded in erect part of colony
G. Polypides	"seen only at ends of filaments"—Goddard, so probably were short	short and stubby; at tips of zooecial tubes	short and stubby	sometimes arc-shaped when retracted
H. Source of above information	Goddard, 1909	Rogick, present study	Abricossoff, 1927b	Borg, 1937

Previous authors have given ample data on the tentacle number of *F. sultana.* Allman (1856, p. 112) states that this species has about 24 tentacles. His Plate IX, Figure 2, shows 20 to 24 tentacles on various polypides while his Figure 7 (same Plate) shows 25. Nowhere does he call attention to this large number however. Hyatt's (1868, p. 220) *F. regina,* now a synonym for *F. sultana,* had 18 to 22. Kraepelin's (1887, pp. 92, 103) specimens had 20 to 22 as a rule but could also range from 18 to 24. Braem's (1890, p. 11) ranged from 20 to 22, with one specimen being found which had only 17. Toriumi's (1941, pp. 196-197) had 17 to 23. The present writer has found New Rochelle specimens with 24 (1940, Study IX, p. 195), Lake Erie specimens showing the full range of 18 to 24, but usually with 20 to 22 tentacles (1935, Study II, p. 250).

Borg (1937, pp. 272-275) reported the collection of a *F. sultana,* from the Sahara region of Africa, which had 24 to 28 tentacles, wider zooecial tubes than the

---

EXPLANATION OF PLATE I

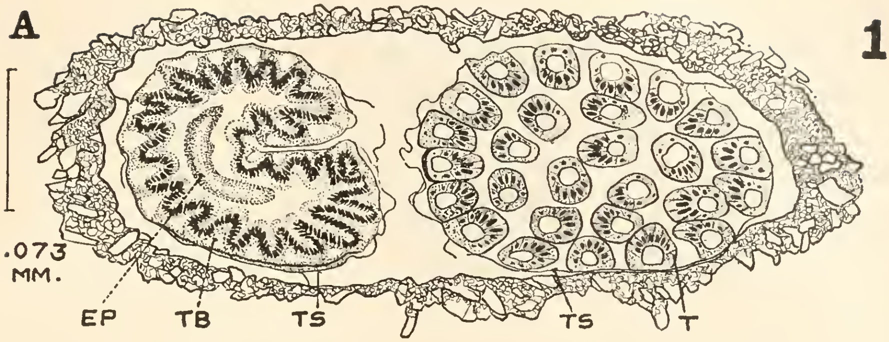
All figures are of *Fredericella australiensis* var. *browni* from the Wyoming collection and have been drawn with the aid of a camera lucida.

FIGURE 1. Cross section through a sand and debris incrusted zooecial tube near the tip, which at this level contains the retracted tentacular crowns of two polypides. The tentacular crown at left has been sectioned through the lophophore region at the bases (TB) of the tentacles and through the epistome (EP). The lophophore bears 25 tentacles in this specimen and their bases (TB) at this level are somewhat triangular. The heavy staining of the nuclei accounts for the darkest wavy "stratum" of the tentacles. The lightly stippled material immediately on either side of this dark nuclear "line" or "band" is cytoplasmic material. At this level the surface of the tentacles facing the epistome is ciliated but that is not shown on the drawing. The tentacular crown at left appears to be horseshoe-shaped but that is because it is in the retracted condition. Such a condition also occurs in a retracted *F. sultana* polypide (see Braem, 1890, Pl. V, Fig. 68). The group of 27 tentacles (T) at right belongs to a second polypide. The tentacular cell nuclei are more conspicuous on the inner border of each tentacle where the cells are taller and closer together than on the outer border where the cells are flatter. By inner border is meant the surface facing the epistome and by outer border is meant the surface at the periphery of the tentacular crown. That orientation is best noted in the outermost circle of tentacles. Those within the circle are less regularly oriented. Here again, the cilia have been omitted from the drawing. The zooecial tube is a somewhat longer ellipse here near the zooecial tip than at a level lower down along the tube, as shown in Figure 10. The wall of the tube varies in thickness because of the incrustation. Drawn to Scale A which is 0.073 mm. long.

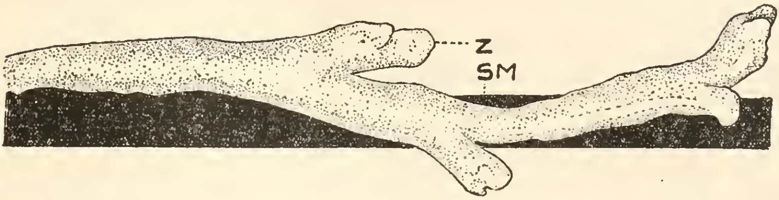
FIGURE 2. A branch from a colony, showing the zooecial tubes (Z) closely adherent to the substratum (SM), which in this instance is blacked in. The tips of the zooecia are not generally attached to the substratum but are free and directed upward. The condition of the tips indicates that all the polypides are retracted. Drawn to Scale C.

FIGURE 3. Six sessoblasts (SS) shown inside the thin, translucent, tubular, cellular or membranous endocyst (EN). The ectocyst has been removed from the specimen. The cement ring is the darkest part of the sessoblast here. Three of the sessoblasts are turned a little so that one edge shows, but the other does not. The endocyst was torn at the right during dissection and the right-hand statoblast is partly out of it. Drawn to Scale B, which is 0.3 mm. long.

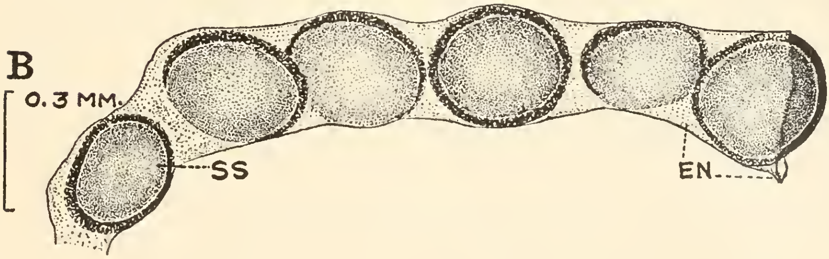
FIGURE 4. Habit sketch of a part of a colony or zoarium showing the adherent base, the upright branches and the mode of dichotomous branching. The substratum is shown in black. When the zooecial tips appear as in this figure their tentacles are either generally retracted or else the tips may be empty. It is sometimes hard to tell if the colony has polypides within it or not because the ectocyst is fairly opaque, so that only very dark structures like the sessoblasts are perceptible with any ease. Since polypide parts are light in color they usually do not show through the ectocyst but have to be dissected out for study. If a colony has been empty a long time the zooecial tips may be broken off and then their emptiness, of course, is evident. Drawn to Scale C which is equivalent to one mm.


A
.073 MM.
EP
TB
TS
TS
T

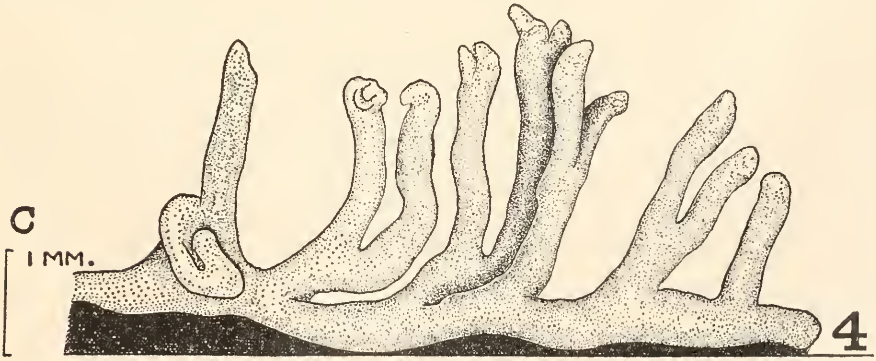
1



2



3



4

PLATE I

ordinary *F. sultana,* and statoblasts which were extremely variable (Table I) and in many cases rounded or oval. Some of his specimens (Borg, 1937, Pl. XVII, Figs. 2 and 3) look very much like *F. sultana* and probably are but his Figure 1 (same plate) appears definitely to belong to *F. australiensis.* Judging by tentacle number, zooecial tube diameter, and appearance of the pictured statoblast inside its tubes, it seems to agree favorably with var. *browni.*

Borg (1937, p. 275) also mentions very incidentally another interesting form of *Fredericella, F. sultana* forma *major,* from the north of Sweden, which has 28 to 32 tentacles and is generally of a greater width (presumably zooecial tube width). This would be in conformity with *F. australiensis.* Unfortunately however, he gives no description, pictures, or dimensions of it so that its status is quite uncertain. It may either prove a new species of *Fredericella* or a new variety of *F. australiensis.* At any rate it would be worth a fuller investigation.

EXPLANATION OF PLATE II

These are all figures of *F. australiensis* var. *browni* (from the Wyoming locality) and were drawn with the aid of a camera lucida.

Figure 5. Surface view of the greater part of one fairly young completed sessoblast. The chitinous substance of the valve gradually thins out toward the center which part is the last to be closed over by the chitin in development. In this specimen the central region was thinnest and palest in color. Drawn to Scale H.

Figure 6. An abnormally shaped sessoblast. There were relatively few mis-shapen sessoblasts found in the collection and this was one of them. Its drawing is included as a contrast to the typical sessoblasts shown in Figures 9 and 11. The sessoblast valves are joined together at the border in what is sometimes called a cement ring (CR). The sessoblast contains opaque germinative material (GM) occupying almost all the space between the two capsule valves. The cement ring is dark amber color while the valves are a paler amber.

Figure 7. A tentacular crown dissected from a zooecial tube, from preserved material. It shows the relative length of the tentacles. The tentacular mass was slightly disarranged during dissection. Drawn to Scale D whose length is given below the figure.

Figure 8. A side or edge view of a sessoblast. The two irregular dark patches (CH) on one valve are chitinous material which grows on some of the sessoblasts, attaching them to the substratum, or to the wall of the colony. A face view of a similar growth is shown in Figure 9. Drawn to the same scale as Figures 9 and 11.

Figure 9. A portion of the cellular endocyst tube (EN) enclosing a sessoblast (SS) on which are growing several irregular or crescent-shaped patches of chitin (CH). The sessoblast is typical, normal. Drawn to Scale E.

Figure 10. A cross section of a zooecial tube taken about midway between the tip and the base, shown in silhouette. This section is more typical of the elliptical shape of the ectocyst tube than is Figure 1, which was taken near the tip which housed the broadest part of the polypides. The irregularity of the zooecial wall is due to the material incrusting it (see Figs. 1 and 14). Drawn to Scale F.

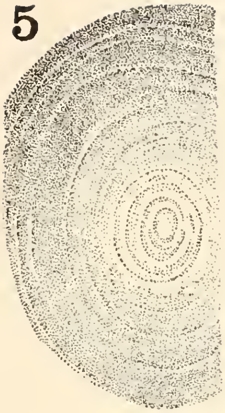
Figure 11. A sessoblast showing the internal germinal mass (GM) shining through the deep amber-colored translucent capsule. The colors of the rest of the sessoblast at the line of junction of the two valves are as follows. The outermost stippled ring is dark reddish amber while the ring shown in black is a very dark brown. These two dark outer bands represent the cement ring area. The shape of the sessoblast is typical for this variety and species. Drawn to Scale E.

Figure 12. Surface view of a portion of a sessoblast valve which is older than that portrayed in Figure 5. A delicate raised chitinous tracery, here shown in black, covers it. Drawn to Scale G.

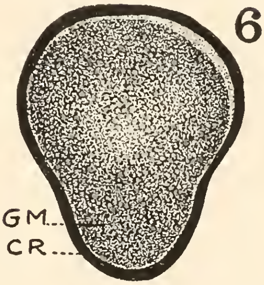
Figure 13. Surface view of a portion of still older sessoblast valve than shown in Figure 12. The raised tracery is coarser, darker, and more prominent. Drawn to Scale G.

Figure 14. Surface view of ectocyst showing the minute sand grains and other debris imbedded in it. Drawn to Scale H.

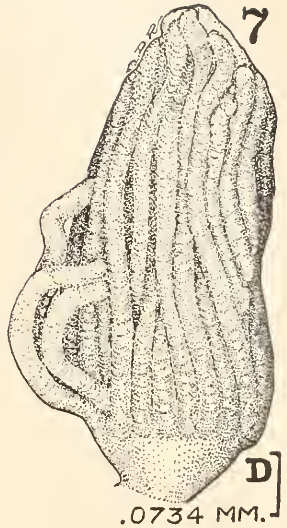
5



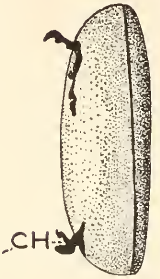
6



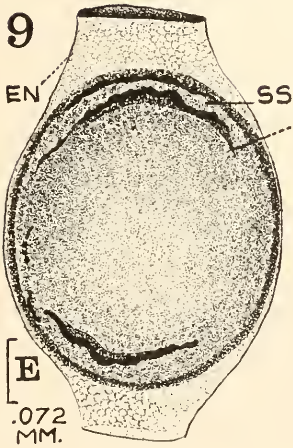
7



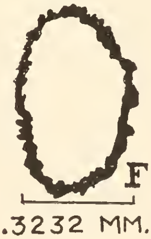
8



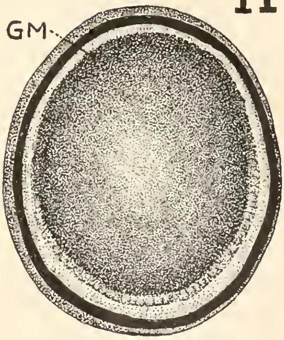
9


EN
SS
E
.072 MM.

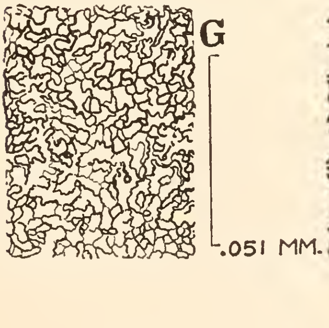
10



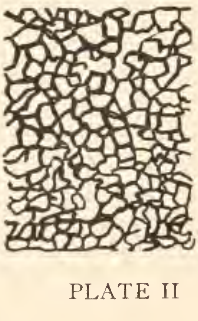
11



12



13



14

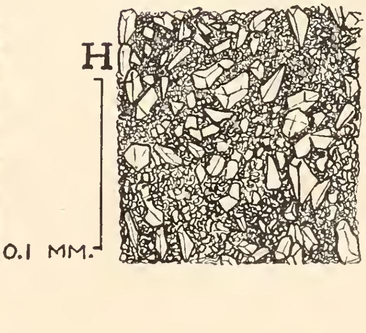


PLATE II

Borg mentions that Kraepelin (1914, reference not available to present author) has collected specimens of *Fredericella* from Rhodesia, Africa, which have statoblasts which are about one third smaller than ordinary German *F. sultana* specimens. Nothing is said about the number of tentacles in the Rhodesian form.

The shape of the expanded tentacular crown in *F. sultana* is nearly circular. In *F. australiensis* var. *australiensis* the lophophore is very definitely elliptical in shape, measuring 0.23×0.38 mm. In var. *browni,* it can not be said for certain what the shape is in expanded lophophores since all were retracted in the material studied. Abricossoff makes no mention of this point in var. *transcaucasica.* Cross sections of retracted *F. sultana* and *F. australiensis* look similar except that the latter species has a greater number of tentacles. When the polypides of both species are withdrawn, their lophophores assume a crescent or horseshoe shape (Fig. 1 of present study; Braem, 1890, Pl. V, Fig. 68; Goddard, 1909, p. 491 and Pl. XLVII, Fig. 5).

Sessoblasts

Statoblasts are extremely important in identification of fresh-water Bryozoa, but those of *Fredericella, Plumatella,* and *Hyalinella* often are not entirely adequate in themselves, especially when present in very small numbers, to determine the exact variety or sometimes even the species to which they belong. It is necessary that sufficient specimens be available so that the normal type of statoblast can be observed, for there is so much variation in shape and size that one can readily be misled by examination of just one or two lone statoblasts. There is a great amount of intergradation between statoblasts of different varieties and species. Almost every worker has rather helplessly commented on the fact, yet has been unable to find a criterion that is invariable by which to identify the species and varieties. Statoblasts alone of the above forms are often insufficient for absolute identification. One should also have the colonies and polypides, living and preserved, in sufficient quantity to really make accurate identifications.

In *Fredericella* there is apparently a complete series of intergrading sessoblasts between *F. sultana* and *F. australiensis.* However, the vast majority of the *F. sultana* statoblasts are reniform or quite elongated while the majority of the *F. australiensis* sessoblasts are more rounded or broadly elliptical in outline.

The extreme dimensions for *F. sultana* sessoblasts are: length range from 0.27 to 0.57 mm. and width range from 0.139 to 0.37 mm. The minimal figures above are from some Lake Erie specimens (Rogick, 1935, Study II, p. 250) and the maximal figures are for some European specimens (Kraepelin, 1887, p. 104). As a rule, the average length and width figures show that *F. sultana* sessoblasts are considerably longer than wide, a fact that can not always be fully appreciated from lone maximum and minimum figures. The extreme dimensions, so far determined for *F. australiensis* sessoblasts, are: length range from 0.331 to 0.470 mm., width range from 0.267 to 0.367 mm. if var. *browni* and var. *transcaucasica* (Tables I and II) are considered, or 0.22? to 0.367 if Dr. Borg's African specimens are included in these computations and if the African forms should all prove to belong to F. *australiensis* and not to *F. sultana.* The reason for the question mark after 0.22 in the preceding sentence is that this particular measurement may or may not have been of this species or variety. The average length and width of *F. australiensis* statoblasts,

TABLE II

Measurements of Fredericella australiensis var. browni from Wyoming

Part or structure	Maximum	Minimum	Average	Number of readings
A. Sessoblast				
1. Total length	0.461 mm.	0.331 mm.	0.382 mm.	69
2. Total width	0.367 mm.	0.266 mm.	0.316 mm.	69
3. Thickness in middle			0.101 mm.	1
4. Cement ring diameter			0.014 mm.	1
B. Zooecial tube diameter along the longer of the two transverse axes	0.576 mm.	0.259 mm.	0.391 mm.	50
C. Tentacles				
1. Number	28	24	26–27	26
2. Length	0.514 mm.	0.383 mm.	0.451 mm.	3
3. Broadest part of the shorter transverse diameter	0.029 mm.	0.020 mm.	0.025 mm.	10
4. Longer transverse diameter (at right angles to preceding measurement)	0.027 mm.	0.019 mm.	0.024 mm.	14
D. Lophophore retracted within zooecial tube:				
1. Antero-posterior diameter	0.308 mm.	0.147 mm.	0.182 mm.	8
2. Lateral diameter	0.170 mm.	0.111 mm.	0.133 mm.	8
E. Epistome				
1. Antero-posterior diameter			0.019 mm.	1
2. Lateral diameter			0.056 mm.	1
F. Esophagus				
1. Length			0.193 mm.	1
2. Width	0.060 mm.	0.051 mm.	0.054 mm.	3
G. Stomach				
1. Length	0.653 mm.	0.634 mm.	0.644 mm.	2
2. Width	0.070 mm.	0.066 mm.	0.068 mm.	2

at least of the *browni* variety, show that the statoblasts are more nearly a broad ellipse than are those of *F. sultana.* The *F. australiensis* sessoblasts are generally slightly flattened on one side and very probably roughened by various markings on the other, when mature (Figs. 12 and 13). Neither Goddard nor Abricossoff mention the nature or pattern of the surface markings on their specimens' sessoblasts. Variety *browni* however had some sessoblasts with markings (Figs. 12 and 13); so does *F. sultana* (Rogick, 1937, p. 102, Fig. 1).

Distribution

Fredericella australiensis has a widely scattered distribution although it has been reported relatively few times. Its three varieties are distributed as follows. Variety *australiensis* occurs in the water supply system at Pott's Hill in New South Wales, Australia (Goddard 1909, pp. 487-489). Goddard reported that the *F.*

sultana recorded earlier from Australia by Whitelegge is probably his own *F. australiensis.* Variety *transcaucasica* occurs in Lake Madatapeen, Tiflis District, the Transcaucasus, in the U.S.S.R. (Abricossoff 1927a, p. 308 and 1927b, p. 91). This variety was collected by B. S. Winograd on July 1, 1915 and later identified by Dr. Abricossoff. Variety *browni* occurs in Uinta County, Wyoming, U.S.A. Some of Dr. Borg's material from rivers in the Sahara region of North Africa is very likely *F. australiensis* var. *browni.* This widens the distribution of *F. australiensis* to 4 ? continents: Africa?, Australia, Eurasia, and North America.

Key to Varieties of Fredericella australiensis

1 (2) Chitinous ectocyst well incrusted with sand grains and debris; rather opaque...........3
2 (1) Chitinous ectocyst very little incrusted; very transparent; zooecia about 0.5 mm. wide; sessoblasts average 0.315 × 0.47 mm................................var. *transcaucasica*
3 (4) Tentacle number 24–28; sessoblast average 0.316 × 0.382 mm.; zooecial tubes elliptical in cross section..var. *browni*
4 (3) Tentacle number 28–30; zooecial tubes roughly triangular in cross section var. *australiensis*

FREDERICELLA AUSTRALIENSIS VAR. BROWNI, NEW VARIETY

Description and Discussion

This variety is illustrated in Figures 1 through 14. Its measurements are given in Table II. Its points of difference and resemblance as compared with the other two varieties are briefly summed up in Table I. Some gaps exist in the information about this variety and they are: 1, the shape and dimensions of the expanded lophophore and 2, the unavailability of living specimens for a more complete study of tentacle and polypide size and variation. However, on the basis of the preserved material available, the following description of the variety can be made.

Variety *browni* has a thin chitinous ectocyst well incrusted with sand grains and debris (Figs. 1 and 14). It is of light tan color and rather opaque. The opacity of the zooecia is such that it is possible to see whether the much darker colored sessoblasts are present, but not whether polypides are present because the light color of the polypides blends in so well with the color of the incrusted ectocyst. To determine if tubes contain polypides it is frequently necessary to tear them apart. Only then are the polypides visible.

Basal zooecia are recumbent or adherent in their more proximal part, with the tips directed upwards (Figs. 2 and 4). From these arise erect branches (Fig. 4). The zooecia are generally elliptical in outline (Figs. 1 and 10). Occasionally a faint keel may be present (Fig. 2) but usually it is not noticeable. The colony appears upon rocks as a coarse tracery or tufted mass, depending upon the number of polypides in it. If the number of polypides is small or if the periphery of the colony is examined there will be located the more adherent members. If the colony is luxuriantly branched and on a rather limited substratum then it has many more upright branches. These are not fused together but retain their individuality and open mode of branching. The zooecia are usually very long (Fig. 4). The ectocyst has considerable rigidity and firmness. The zooecia are somewhat wider than in *F. sultana.* Those of var. *browni* are not as wide apparently as those of var. *transcaucasica* (Table I). The ectocyst is too opaque to be able to see dissepiments or incomplete septa at the commencement of the zoids even if they were present in this variety. Such dissepiments occur in *F. sultana.* A diligent search was made through sectioned and dissected *F. australiensis* var. *browni* material but no dissepiments could be found.

The ectocyst is lined with a soft thin transparent membranous endocyst. The endocyst encloses the polypides and sessoblasts (Figs. 3 and 9).

The polypides of var. *browni* appear short and stubby. The tentacles, especially, seem so, perhaps because of their considerable number, 24–28 (Fig. 7). The tentacles ranged in number from 24 to 28 but the usual number was 26 or 27, just as Borg had found in his African specimens. Of course, the condition of the colony, the length of the polypides and tentacles are greatly influenced by the state of nutrition of the colony. The better fed the colony, the longer the polypides and tentacles. However, the var. *browni* specimens seemed well enough nourished. Their digestive tracts were well filled with algal food.

The parts of the digestive tract are the same as for *F. sultana* and *Plumatella repens*—ciliated mouth guarded by the epistome, ciliated pharynx, esophagus, stomach, and intestine.

The reproductive organs were not observed.

The sessoblasts of var. *browni* are generally smooth on one side (Fig. 5) and roughened on the other (Figs. 12 and 13). However, some older sessoblasts may show roughening or markings on both sides, and in addition, chitinous material may begin to grow on the valve of the statoblast (Fig. 8), attaching it to the endocyst (Fig. 9) or to the body wall and possibly eventually to the substratum.

Variety *browni's* sessoblast shape is best shown in Figures 9 and 11, which are typical. Abnormal specimens occasionally occur and one such is shown for contrast in Figure 6.

The colors of the sessoblasts range from reddish yellow to brown, depending upon the age; the older, the darker.

There were quite a number of sessoblasts present in the zooecial tubes of the Wyoming specimens at the time of collection (August).

The sessoblasts were so distinctive in shape and general proportions that it was immediately evident that one was not dealing with *F. sultana* but with a form related to Abricossoff's and Goddard's specimens — a distinct species — *F. australiensis.*

The decision to make each of these forms (*F. australiensis, F. sultana transcaucasica,* and the Wyoming specimens) a separate variety of *F. australiensis* was based on the great similarity to each other so far as the shape of their statoblasts was concerned and their slight but distinct differences as regards the nature of the ectocyst and the number of tentacles (refer to Key to Varieties and Tables I and II).

SUMMARY

1. The species *Fredericella australiensis* has been emended to include three varieties.

2. A new variety, *F. australiensis* var. *browni,* has been erected.

3. Two other previously recorded forms, *F. australiensis* Goddard 1909 and *F. sultana* subsp. *transcaucasica* Abricossoff 1927 have been reduced to the status of varieties under the emended *F. australiensis.*

4. The emended *F. australiensis* is characterized by its rounded or broadly

elliptical sessoblasts, its wider zooecial tubes, its greater tentacle number, its lack of dissepiments and the shorter stubbier tentacles and polypides which are generally confined to the tips of the tubes. These features distinguish it from *F. sultana.*

5. The varieties *australiensis, browni,* and *transcaucasica* are placed in *F. australiensis* because they possess the above characteristics.

6. The three varieties are distinguished from each other on the basis of degree of incrustation of their ectocyst, the difference in number of tentacles, appearance of the zooecial tubes in cross section and miscellaneous measurements.

7. *Fredericella australiensis* has a wide but scattered distribution. It is represented in Australia by var. *australiensis*; in Eurasia (the U.S.S.R.), by var *transcaucasica,* in Africa?; and in North America, by var. *browni.*

8. The specimens which were immediately responsible for the erection of the new variety, *F. australiensis* var. *browni,* were obtained through the kindness of Dr. C. J. D. Brown and Dr. H. van der Schalie of Ann Arbor, Michigan, who turned the collection over to the author for study. The specimens were collected by Dr. van der Schalie on August 3, 1942, from rocks in an alkali pond about three miles northeast of Church Butte, Uinta County, Wyoming, U.S.A.

9. The study includes 14 illustrations and one table of measurements dealing with var. *browni* and one table of comparison between the three varieties.

10. A brief summary of available measurements and other data on *F. sultana* is given.

LITERATURE CITED

Abricossoff, G., 1927a. Über die Susswasser-Bryozoen der USSR. *Compt. Rend. d l'Acad. Sci. de l'URSS.,* **1927**: 307–312.

Abricossoff, G., 1927b. To the knowledge of the fauna of the Bryozoa of the Caucasus. *Russ. Hydrobiol. Zeitschrift, Saratow, USSR,* **6** (3/5): 84–92.

Allman, G., 1856. A monograph of the fresh-water Polyzoa, including all the known species, both British and foreign. *Ray. Soc., London,* 120 pp., 11 Pl.

Borg, F., 1937. Sur quelques Bryozoaires d'eau douce Nord-Africains. *Bull. Soc. d'Hist. Nat. de l'Afrique du Nord,* **27** (7): 271–283.

Braem, F., 1890. Untersuchungen über die Bryozoen des süssen Wassers. *Bibliotheca Zoologica,* Heft **6**. 154 pp., 15 Pl.

Goddard, E. J., 1909. Australian freshwater Polyzoa. Part 1. *Proc. Linn. Soc. N. S. W.,* **34**: 487–496. 1 Pl.

Harmer, S. F., 1913. The Polyzoa of Waterworks. *Proc. Zool. Soc. London,* **1913**: 426–457.

Hyatt, A., 1868. Article X. Observations on Polyzoa, Suborder Phylactolaemata. *Comm. Essex Inst.,* **5**: 193–232.

Kraepelin, K., 1887. Die deutschen Süsswasserbryozoen. Eine Monographie. I. Anat.-Syst Teil. *Abhandl. d. naturw. Vereins Hamburg,* **10**: 1–168.

Kraepelin, K., 1914. Bryozoa. *Beiträge z. Kennt. d. Land u. Süsswasserfauna Deutsch-Südwest-Afrikas. Ergebn. d. Hamburger Deutch-Südwest-Afr. Studienreise, 1911.* (Ref. not available to present author.)

Rogick, M. D., 1935. Studies on fresh-water Bryozoa, II. *Trans. Amer. Micr. Soc.,* **54** (3): 245–263.

Rogick, M. D., 1937. Studies on fresh-water Bryozoa, V. *Ohio Jour. Sci.,* **37** (2): 99–104.

Rogick, M. D., 1940. Studies on fresh-water Bryozoa, IX. *Trans. Amer. Micr. Soc.,* **59** (2): 187–204.

Rogick, M. D., 1943. Studies on fresh-water Bryozoa, XIV. *Annals N. Y. Acad. Sci.,* **45** (4): 163–178. 3 Pl.

Rogick, M. D., 1945. Studies on fresh-water Bryozoa, XV. *Ohio Acad. Sci.,* **45** (2): 55–79.

Toriumi, M., 1941. Studies on fresh-water Bryozoa of Japan, I. *Sci. Repts. Tôhoku Imper. Univ. (4: Biol.),* **16**: 193–215.

STUDIES ON THE BIOCHEMISTRY OF TETRAHYMENA. VII. RIBOFLAVIN, PANTOTHEN, BIOTIN, NIACIN AND PYRIDOXINE IN THE GROWTH OF T. GELEII W

GEORGE W. KIDDER AND VIRGINIA C. DEWEY[1]

Arnold Biological Laboratory, Brown University, Providence, R. I.

With the substitution of chemically known materials for all but one fraction in the medium for the growth of Tetrahymena it has been possible to determine with some degree of exactness the specific vitamin requirements of this important ciliate. When proteins, such as casein or gelatin, or peptones are used as the base medium it has been impossible to determine the importance of those vitamins which were stable to treatments which would not also destroy other essential materials. Using these types of media, claims have been made for the essential nature of thiamine and of riboflavin for *Tetrahymena geleii* (Hall and Cosgrove, 1944; Hall, 1944). It was earlier indicated (Kidder and Dewey, 1942) and later conclusively proven (Kidder and Dewey, 1944; 1945a; 1945b) that at least eight strains of Tetrahymena could grow in a medium in which the thiamine had been destroyed.

When it was found that *T. geleii* could be grown successfully in a mixture of amino acids (Kidder and Dewey, 1945c) and that two of the three "unknown growth factors" could be replaced with nucleic acid derivatives (Kidder and Dewey, 1945d) and that the remaining "unknown growth factor" (Factor II) was relatively stable and was not adsorbed readily on activated charcoal, it became possible to examine the effects of the omission of a number of the vitamins. Hitherto these vitamins had been added routinely to guard against the possibility of any one of them proving to be a limiting factor. It was found (Kidder, 1945) that folic acid is an essential growth factor for *T. geleii* W, this fact being obscured previously by the necessary inclusion of Factor I (containing folic acid) as the lead acetate precipitate fractions of raw materials, the Factor I activity being readily absorbable on activated charcoal.

The present work has been made possible by the utilization of a number of different treatments of the Factor II preparations and the inclusion of all other constituents of the medium as chemically pure materials. Furthermore, this work would not have been possible without the employment of a microbiological method for the detection of traces of the growth factors under consideration. We have utilized *Lactobacillus casei* as a tool in this study, and while we have made no attempts to assay various preparations quantitatively, we have used the bacterial method for determining the total lack of the vitamin under immediate consideration. It has been possible also, to show that the ciliate possesses the ability to synthesize certain of the B vitamins, by determining the increase of the vitamin by the *L. casei* test after the growth of the ciliate.

---

[1] Aided by grants from the Morgan Edwards Fellowship Fund and the Manufacturers Research Fund for Bacteriology and Protozoology of Brown University. Present address Biological Laboratory, Stanford University.

MATERIALS AND METHODS

Organisms

The ciliate used in this study was *Tetrahymena geleii* W, which has been maintained in pure (bacteria-free) culture in this laboratory for a number of years and which has been used in numerous previous studies (Kidder and Dewey, 1942–1945). The organism has been grown in amino acid media for the past one and one-half years and all inocula for the present series were taken from these stocks.

Lactobacillus casei 912 was used for the microbiological testing of experimental media. This organism was obtained from the Squibb Institute for Medical Research through the courtesy of Dr. Vincent Groupé. Stocks were carried in yeast extract-dextrose-agar stab cultures, transplants being made at monthly intervals, incubated at 37° C. for 24 hours and then placed in the refrigerator.

Ciliate base medium

One type of base medium was used routinely. This appears in Table I with the complete supplements. Each vitamin under investigation was omitted from the medium separately.

Preparation of Factor II

It has been necessary to treat the Factor II preparations in various appropriate ways in order to eliminate the different vitamins studied. In the earlier work (Kidder and Dewey, 1945d) the prime consideration in the Factor II preparation was the elimination of Factors I and III activity, and the methods used did not necessarily render the preparation vitamin free. In this study the inclusion of Factor I and Factor III activity was of no particular importance, and so attempts were made to eliminate the vitamin under consideration and still retain maximum Factor II activity. This latter was not always possible as some of the treatments used not only removed or destroyed the vitamin but also lowered the Factor II activity. Nevertheless preparations which were satisfactory for this study were obtained, and these will be described under the heading of each vitamin.

Riboflavin-free preparation (SL531).

Liver Fraction L [2] (50 grams) was dissolved in one liter of distilled water and a 40 per cent solution of normal lead acetate was added until no more precipitate formed. The precipitate was removed by filtration with the aid of Celite and discarded. The filtrate was neutralized with NaOH and treated with an excess of basic lead acetate. The second precipitate was removed and discarded, the excess lead removed with 9 per cent oxalic acid and the excess oxalic removed as the oxalate with $Ca(OH)_2$. Tests at this stage showed the presence of large amounts of riboflavin, but after adsorption with 10 grams of Norit A for one hour at room temperature at pH 3.5 the riboflavin had been quantitatively removed. This preparation was used in a concentration of one part in ten parts of final medium.

---

[2] Furnished through the courtesy of Dr. David Klein and the Wilson Laboratories.

TABLE I

Base Medium

	mg./ml		micrograms/ml.
l(+)-arginine mono-hydrochloride	0.82	biotin methyl ester[3]	0.00005
l(−)-histidine mono-hydrochloride	0.10	calcium pantothenate[3]	0.10
dl-isoleucine	0.35	thiamine hydrochloride	0.10
dl-leucine	0.35	nicotinamide[3]	0.10
dl-lysine	0.60	riboflavin[3]	0.10
dl-methionine	0.34	pyridoxine hydrochloride[3]	0.10
dl-phenylalanine	0.14	*p*-aminobenzoic acid	0.10
dl-serine	0.04	*i*-inositol	1.00
dl-threonine	0.20	choline chloride	1.00
l(−)-tryptophane	0.10	folic acid concentrate[4]	1.00
dl-valine	0.20		
dextrose	2.00		mg./ml.
$MgSO_4.7H_2O$	0.10	hydrolyzed yeast nucleic acid[5]	0.05
K_2HPO_4	0.10		
$CaCl_2.2\ H_2O$	0.05	Factor II preparation (see text)	
$FeCl_3.6\ H_2O$	0.00125		
$MnCl_2.4\ H_2O$	0.00005		
$ZnCl_2$	0.00005		

Pantothen-free preparation (8L531H).

Although pantothenic acid is adsorbed on activated charcoal the time and temperature allowed in preparing the riboflavin-free medium is insufficient for the complete removal of pantothen. Raising the temperature, increasing the time, or increasing the amount of Norit used was not practical as the Factor II activity was greatly reduced (Kidder and Dewey, 1945d). Therefore advantage was taken of the sensitivity of pantothenic acid to alkali and heat and the riboflavin-free preparation was adjusted to pH 10.0 with NaOH and autoclaved for two hours. The Factor II activity was somewhat reduced by this treatment, but the preparation was entirely satisfactory for use. *L. casei* tests showed that the pantothenic acid content had been lowered to an insignificant amount. This preparation was used in concentrations of one part in ten parts of final medium.

Biotin-free preparation (8L5C1)

The most active biotin-free preparation, and therefore the one used in this study, was made in the following manner. Ten grams of Liver Fraction L was dissolved in 200 ml. of distilled water and brought to boiling. To this boiling mixture were added 10 ml. of a 10 per cent solution of $NaHSO_3$ and 10 ml. of a 10 per cent solution of $CuSO_4$, and boiling was continued for 3–5 minutes. The precipitate was removed on a fluted filter and the process repeated once. The copper was removed as CuS after treating with 15 per cent Na_2S and the sulfate and sulfite removed as the barium salts after treatment with $Ba(OH)_2$. The volume of the filtrate was reduced to 200 ml. and a 100 ml. aliquot was adjusted to pH 3.5. Two grams of Norit A was added and adsorption allowed to continue for one hour at room temperature with constant stirring. This preparation was used in a concentration of one part in twenty parts of final medium.

---

[3] Omitted singly in the appropriate series of experiments.

[4] The folic acid concentrate used had a potency of 5000 and was furnished through the courtesy of Dr. R. J. Williams.

[5] Assays of the hydrolyzed yeast nucleic acid with *L. casei* showed it to be free of riboflavin, pantothen, biotin, niacin, and pyridoxine but appreciable amounts of folic acid were present.

Niacin-free preparation (8L5C2)

The use of copper precipitation, described above, was designed for the removal of nicotinic acid. While most of the niacin activity was removed by this method, as shown by the *L. casei* test, enough remained to warrant further treatment. Accordingly the filtrate from the copper precipitation was extracted with *n*-butanol for 96 hours in a continuous extraction apparatus (Wilson, Grauer, and Saier, 1940). It is known that nicotinamide is readily extracted with butanol, and after this treatment the extract was found to be entirely devoid of niacin activity, even when tested with *L. casei* in amounts four times greater than those used as a supplement for the ciliate. This preparation was used in a concentration of one part in twenty parts of final medium.

Pyridoxine-free preparation (8L531L)

This preparation was the least successful of any used. While it was possible to treat crude extracts and various filtrates in ways which would remove all pyridoxine activity for *L. casei,* it was usually found that the Factor II activity was also lowered to a point where the preparation was very inferior as a ciliate supplement. Therefore, the most satisfactory preparation, and the one finally used, was very low in Factor II activity, and the results obtained cannot be compared directly with those of the other vitamins tested. This preparation was made by exposing an alkaline lead acetate filtrate fraction (8L531), to direct illumination from a 300 watt electric bulb at a distance of 8 inches for a period of 72 hours. This method was used by Hochberg et al (1943) for pyridoxine destruction. Besides the destruction of appreciable amounts of the Factor II, another disadvantage of the technique was the excessive evaporation which took place during the treatment. It was necessary to add distilled water at frequent intervals to prevent the preparation from drying down. This preparation was used in a concentration of one part in ten parts of final medium.

Assay procedure

The base medium employed for the testing of the various preparations was the 16 amino acid mixture suggested by Hutchings and Peterson (1943). This was chosen in preference to the casein hydrolysate medium of Landy and Dicken (1942) because of the known composition of the former and the fact that lower blanks can be obtained. While the amino acid medium does not permit the production of as much acid by the bacteria it is very satisfactory for determining the presence or absence of a known vitamin.

Because of the scarcity of amino acids we have modified the usual procedure. The amino acid medium is made up for stock in double strength and the sugar, acetate, salts, purines and pyrimidine are added in double strength. For a test, this complete base medium is measured into 125 × 7 mm. Pyrex tubes in one ml. volumes. The material to be tested is added in appropriate amounts and a mixture of the vitamins, minus the one for which the preparation is being tested, is added. The volume is then made up to 2 ml. with distilled water. Two controls were run with each test, one containing base medium and a complete set of supplements except for the vitamin under test. The second control contained the base medium plus the complete supplement and plus the Factor II preparation. The first served as a control on carry-over growth. The second was a control on the possible toxicity of the Factor II preparation. When titrations were made the figure from the first control was subtracted from the figure from the unknown preparation. Inasmuch as a small volume of medium was used it was found advantageous and more accurate to reduce the standard hydroxide to 0.05 N. The NaOH was standardized with 0.05 N oxalic acid, and the amount of acid produced after 96 hours of growth at 37° C. was titrated directly, using brom thymol blue as an indicator. The longer incubation period was used for maximum acid production, for in this way the test becomes more sensitive for traces of vitamins.

After many trials, the usual drop method of inoculation of *L. casei* was abandoned in favor of inoculating with a straight needle. This eliminates the necessity for washing the bacteria and blanks are just as low. The inocula were always made from yeast extract cultures which had incubated for 18–24 hours at 37° C.

While standard curves, using this method, have been made for all the vitamins studied the results obtained with our preparations do not permit quantitative statements as to amounts inasmuch as the tests were always made at very high levels and stimulatory materials in the Factor II preparations were invariably present. We were interested, moreover, first in the determination of the vitamin-free condition of our media, and second, in the biosynthesis of the vitamins by the ciliates. In the latter case, assays were employed on the medium before and after ciliate growth and the difference of acid production between the two compared directly.

It has been pointed out (see Cheldelin et al, 1942) that many of the B vitamins occur in a bound form in tissues and must be liberated by some means for satisfactory tests. There was the possibility that bound vitamins in the Factor II preparations might be available for the ciliate but not for *L. casei*, and these would invalidate any conclusions which were based on the vitamin-free nature of the preparation by the *L. casei* test. Enzymatic digestion was carried out on all the preparations, therefore, in order to test for the total vitamin content. Accordingly takadiastase and pepsin in quantities of one per cent each of the total solids of the preparation to be tested were used. The preparation was allowed to digest under toluene for 24 hours at 37° C. at pH 3.5. After steaming, the digest was added to the assay tubes, as described above, and a control of equivalent amounts of the enzymes added to parallel tubes. This latter control is obviously necessary as the enzymes are not vitamin-free. Data on the results of assays of the Factor II preparations used are presented in Table II.

Ciliate cultures

It was the usual practice, when testing for the effects of one of the known vitamins, to grow the ciliate through at least three serial tube transplants in the medium containing the vitamin being investigated, paralleled with the same medium minus the vitamin. Transplants were made at 72 hour intervals with a bacteriological loop delivering approximately 0.005 ml. of fluid. All incubation was at

25° C. Growth rate was followed by inoculating appropriate amounts of third transplant ciliates (36 hours old) into like media in culture flasks (Kidder, 1941). After inoculation of the flasks (as near 100/ml. as possible) samples were drawn and the initial inoculum determined. Growth thereafter was ascertained by sampling at intervals until the termination of the experiment. In all cases the flask series were repeated at least once and the figures averaged.

TABLE II

Assay of Factor II Preparations with Lactobacillus casei 912

No.	Additions	Vitamin omitted from base medium					
		Riboflavin	Pantothen	Biotin	Nicotinamide	Pyridoxine	Folic acid
1	None	0.05	0.00	0.05	0.49	0.12	0.62
2	Enzyme preparation	0.13	0.38	0.17	0.75	0.10	0.90
3	8L531	0.05	3.79	2.88	4.72	3.86	0.00
4	8L531H	0.03	0.12	2.79	4.80	2.60	0.00
5	8L5C1	0.00	1.53	0.07	0.22	3.10	4.36
6	8L5C2	3.42	0.87	2.93	0.00	3.65	4.65
7	8L531L	0.00	3.74	3.00	4.51	0.09	0.00

Figures represent ml. of 0.05 N acid per culture (2 ml.). All figures corrected for uninoculated blanks. Line 2 corrected for carry-over growth (Line 1). Lines 3–7 corrected for vitamin content of enzyme preparation (Line 2).

One obvious objection to the flask technique is the possibility of introducing the vitamin being investigated from the rubber vaccine tip used in the sampling port. This possibility was diminished by boiling the vaccine tips for one hour previous to setting up the flasks. As a check on the tips uninoculated flasks were manipulated in the same manner as the experimental cultures and the samples tested with *L. casei* for the vitamin being studied. In no case were these detectable amounts of the vitamins present. Sampling needles were made chemically clean as well as sterile before use.

Growth rate during the logarithmic phase was calculated by the formula $g = \frac{t \log 2}{\log b - \log a}$ where t = the time in hours during which the population has been increasing exponentially, a = the number of cells per unit volume at the beginning, and b = the number of cells at the end of time, t.

RESULTS

After obtaining Factor II preparations which were free of the vitamins to be studied, preliminary experiments were set up to determine which vitamins, if any, were essential growth factors for *Tetrahymena geleii* W. Accordingly serial transplants were made in the appropriate media, one set with the vitamin present, and the other with the vitamin omitted. It was immediately apparent that the ciliate lacked all ability to synthesize folic acid (Kidder, 1945) but the absence of none of the other vitamins did more than lower the growth rate and the yield. Growth in

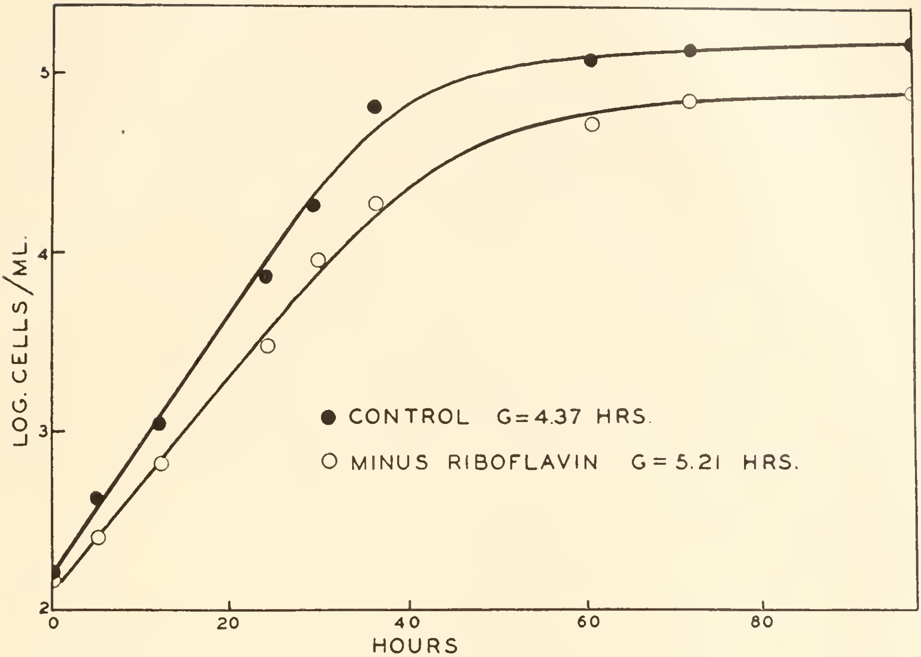


FIGURE 1. Effect of the omission of riboflavin. Factor II preparation used was 8L531. Average of two separate experiments.

TABLE III

Summary of Growth Data

Medium	Generation time in hours	Population per ml. at end of log. phase	Population per ml. at 96 hours
Control	4.37	58,000	164,000
Minus riboflavin	5.21	19,000	67,000
Control	4.57	32,000	90,000
Minus pantothen	4.60	34,500	41,000
Control	4.32	45,500	152,000
Minus biotin	5.01	15,000	96,000
Control	4.17	49,000	170,000
Minus nicotinamide	8.40	7,500	79,000

the sixth serial transplant was possible for all series except that lacking exogenous folic acid.

In order to gain quantitative information regarding the stimulatory effect that was apparent in the tube cultures, growth flasks were inoculated from third transplant tubes and the growth followed by frequent sampling. In the case of pyridoxine, however, the flask cultures were omitted, as the Factor II preparation necessarily used was relatively inactive and the growth was erratic, even when pyridoxine was

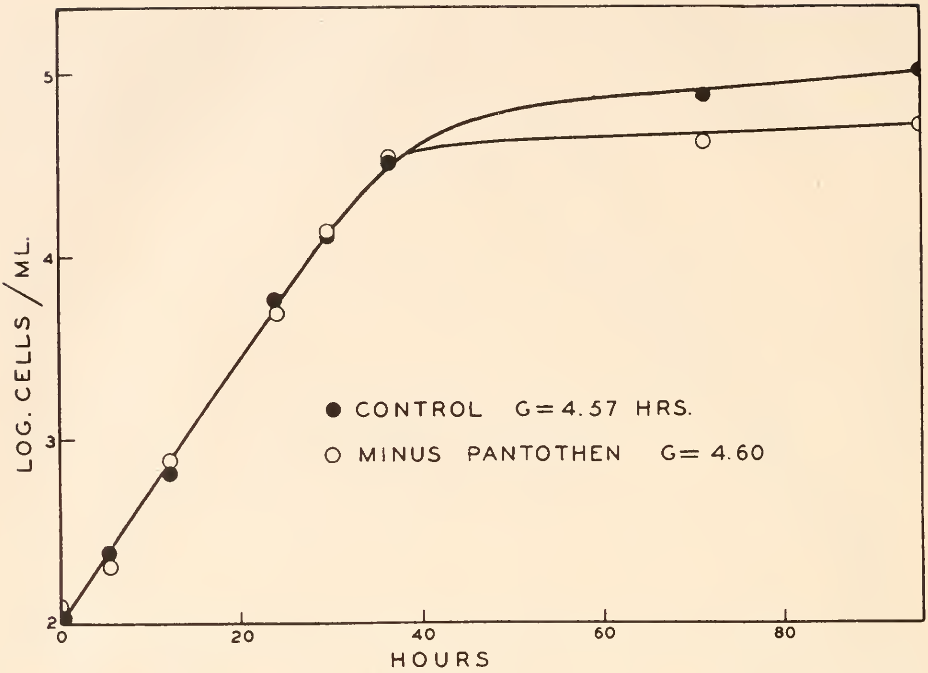


FIGURE 2. Effect of the omission of pantothenic acid. Factor II preparation used with 8L531L. Average of two experiments.

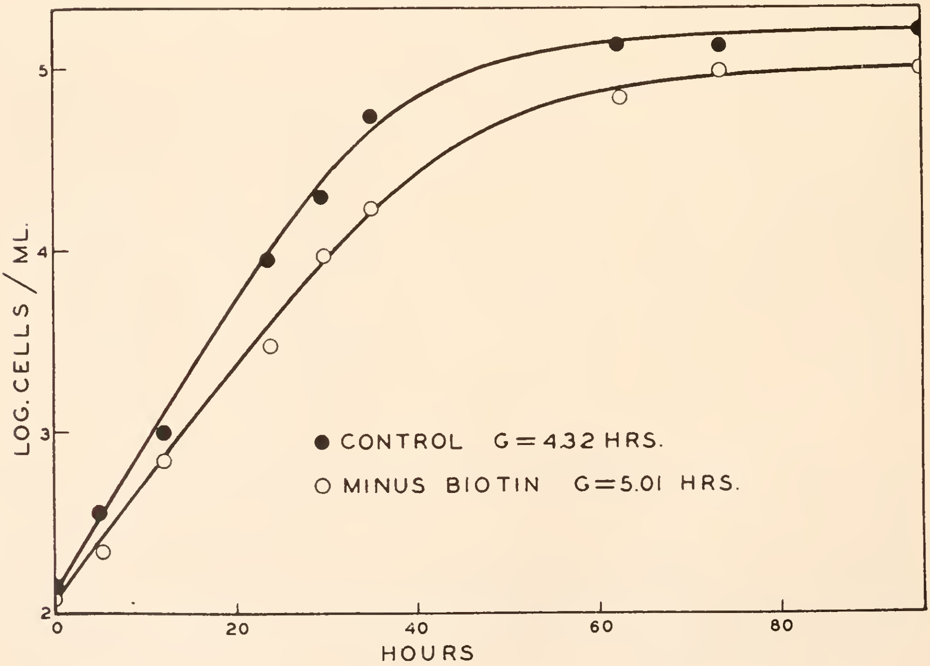


FIGURE 3. Effect of the omission of biotin. Factor II preparation used was 8L5C1. Average of two separate experiments.

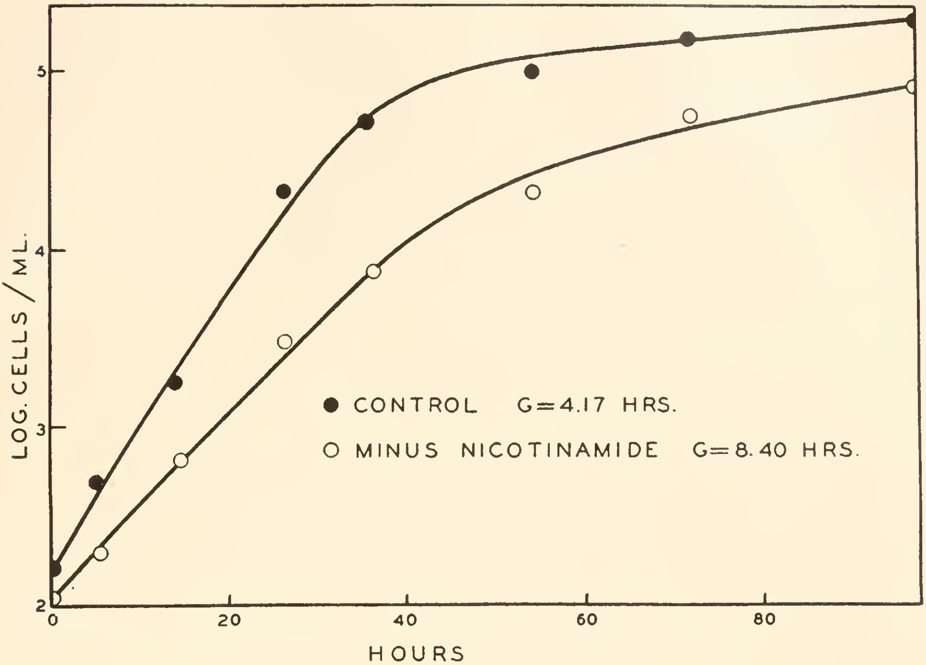


FIGURE 4. Effect of the omission of nicotinamide. Factor II preparation used was 8L5C2. Average of three separate experiments.

present. While qualitative data are lacking for the pyridixine series, nevertheless we can say from the serial tube transplants that this vitamin appears to be only stimulatory for *T. geleii* W.

The omission of riboflavin from the medium resulted in slower growth during the exponential period. Thus the generation time was raised from 4.47 hours in the control flasks to 5.21 hours. The maximum yields were reduced to less than half of those in the control flasks (Fig. 1; Table III).

The ciliates appear to synthesize pantothen at a rate which equals the demands for rapid growth, as judged by the almost identical growth rates in the pantothen-containing and the pantothen-free media (Fig. 2; Table III). In all cases, however, the maximum yield was significantly lower in the pantothen-free cultures.

A comparison of the growth curves, generation times and yields for biotin-free and riboflavin-free media (Figs. 1, 3; Table III) shows remarkable similarity. The rate of synthesis of biotin by the ciliates appears to be low, indicating the stimulatory status of this vitamin. We possess added data on biotin substantiating its non-essential nature for *T. geleii* W. Early in this series of investigations the effect of raw egg white and avidin concentrates were studied as a means of determining whether or not the ciliate required biotin. Egg white was taken aseptically and added to tubes containing 5 ml. of one per cent proteose-peptone, each tube receiving 0.1 ml. According to Eakin, Snell, and Williams (1941), this amount of egg white is enough to inactivate 0.05 micrograms of biotin. The analysis of proteose-peptone made by Stokes, Gunness, and Foster (1944) shows that one gram contains

0.2 micrograms of biotin, hence our tubes each contained 0.01 micrograms of the vitamin. The amount of raw egg white used, therefore, was enough to inactivate five times more biotin than was present. Indefinitely transplantable growth occurred in the proteose-peptone plus egg white. Likewise, the use of avidin concentrates in quantities far in excess of that needed to inactivate all of the biotin present, produced similar results. In this case the avidin was allowed to act on the proteose-peptone, the peptone removed as the diffusate in dialysis, the peptone being used as the medium. Similar results were obtained with proteose-peptone treated with H_2O_2 in a manner similar to that described by Garnjobst, Tatum, and Taylor (1943). While it is clear that biotin is not required by *T. geleii* W this vitamin is stimulatory.

TABLE IV

Assay Data (L. casei) Before and After the Growth of T. Geleii W

Additions for assay (1:10)	Factor II preparation									
	8L531		8L531H		8L5C1		8L5C2		8L531L	
	Plus riboflavin	Minus riboflavin	Plus pantothen	Minus pantothen	Plus biotin	Minus biotin	Plus nicotinamide	Minus nicotinamide	Plus pyridoxine	Minus pyridoxine
Before inoculation	4.78	0.07	3.90	0.17	3.92	0.08	3.88	0.00	3.61	0.16
After 72 hr. ciliate growth. Medium plus cells	4.60	1.18	3.94	1.71	3.90	1.56	3.80	0.21	3.48	0.22
Supernatant of 72 hr. ciliate culture	4.82	0.06	4.13	0.10	3.86	0.10	3.71	0.16	3.52	0.11
Washed ciliates from 72 hr. culture	4.80	1.07	3.94	1.64	3.91	1.05	3.75	0.25	3.61	0.15

Figures represent ml. of 0.05 N acid per culture (2 ml.). All figures corrected for uninoculated blanks and for carry-over growth.

While *T. geleii* W can be transplanted indefinitely in the absence of exogenous nictotinamide this vitamin (or nicotinic acid) is an active stimulant. The generation time is doubled when the ciliate is grown in niacin-free medium as compared to that in the nicotinamide-containing control (Fig. 4; Table III). While the population density at 96 hours is less than one-half that of the control (which is similar to the cases of riboflavin, biotin, and pantothen), the population at the end of the logarithmic phase is extremely low (approximately 7000/ml.).

It was of interest to determine whether or not *T. geleii* W would synthesize amounts of the vitamins which could be detected with the assay methods used. Accordingly the five types of media used above were set up for serial transplants and an aliquot of each was assayed with *L. casei*. After the ciliates had grown for 72 hours in the third transplants, assays were again made for the various vitamins. These assays were of three different types. One was on the whole medium (medium plus cells); one, on the supernatant fluid following centrifugation after chilling (Kidder, Stuart, McGann and Dewey, 1945), and the third was on washed cells equivalent to the concentrations found in the whole medium. The samples to be tested were added to the *L. casei* base medium and sterilized by autoclaving. The results of these experiments are given in Table IV. Appreciable amounts of riboflavin, pantothen, and biotin are synthesized by the ciliates. Increases in amounts of niacin are so small that they probably lack significance and there appears to be no increase in pyridoxine. It must be remembered, however, that the growth in the niacin-free medium is less at 72 hours than in the riboflavin-, pantothen-, or biotin-free media, while the maximum population reached in the pyridoxine-free medium never exceeded 20,000 ciliates per ml. The amounts of the vitamins detected represent minimums, as no attempt was made to release any which may have been bound (except by autoclaving). It is to be noted that all vitamins which were synthesized remained in the cells. This was also found to be true in the case of the biosynthesis of thiamine by *T. geleii* W (Kidder and Dewey, 1942).

Discussion

Due to the various treatments necessary for the removal of vitamins none of the Factor II preparations used in this study produced as high yields as had been previously obtained (Kidder and Dewey, 1945d; Kidder, 1945). While the riboflavin-free preparation was essentially the same as had been used for the study of purines and pyrimidines and of folic acid, variations in potency of Factor II activity were evident. This is due almost entirely to the degree of adsorption on the activated charcoal. Slight variations of temperature appear to effect the degree to which Factor II is lost, so that a critical balance is found between the complete removal of the vitamins and the loss of Factor II activity. In this study the emphasis was placed on the vitamin removal at a sacrifice of yield.

The findings of Hall and Cosgrove (1944) on the importance of riboflavin for their strain of *Tetrahymena geleii* does not seem inconsistent with the present observations. They state that heat—and alkali-treated casein did not support growth unless supplemented with thiamine, and even then poorly. The addition of riboflavin together with the thiamine, however, permitted as good growth as did the casein medium before heating. There can be no doubt as to the stimulatory effect of riboflavin, and it is altogether possible that it may function as a detoxifying agent as well. The detoxifying action of thiamine has been suggested previously in this connection (Kidder and Dewey, 1944).

In addition to the vitamins which have already been investigated for *T. geleii* W there remain at least three of the commonly recognized ones about which little is known. These are *p*-aminobenzoic acid, inositol and choline. As yet we have not had the opportunity to test for the last two, but preliminary work has been started on the first. The commonly employed technique of adding sulfonamides to the medium has indicated that this ciliate requires excessive amounts of the inhibitor to effect growth. The inhibition to growth at these high levels is not completely reversed with *p*-aminobenzoic acid, and the evidence indicates that purines are also involved. This study awaits completion and will be reported at a later date, but it appears that *T. geleii* W may be independent of an exogenous supply of *p*-aminobenzoic acid.

The only other protozoan of animal nature about which there appears to be critical data regarding the requirements of the vitamins studied here is *Colpoda duodenaria* (Tatum, Garnjobst, and Taylor, 1942; Garnjobst, Tatum and Taylor, 1943).

Colpoda requires large amounts of thiamine, pantothen, riboflavin, nicotinamide, and pyridoxine. It does not require *p*-aminobenzoic acid, biotin, or inositol, while the status of choline and folic acid is still unknown. Moreover, Colpoda was shown (Garnjobst, Tatum, and Taylor, 1943) by the *Neurospora* test of Tatum and Beadle (1942) to either release bound biotin from the bacterial "plasmoptyzate" used or to synthesize this vitamin. This biotin appeared in the medium, however, and in this way differs from the condition found with *T. geleii* W where all of the vitamins arising by biosynthesis appear to bound in the cell protoplasm.

The biochemical investigations of *Tetrahymena geleii* W which have so far been completed permit a fairly complete view of its synthetic abilities. Added carbon sources appear to be unnecessary except as they may perform a sparing action on the amino acids. Inorganic salts certainly are essential (Hall and Cosgrove, 1944; Kidder and Dewey, 1944) although the question of which elements need to be included is yet to be determined. The commonly employed inorganic salts usually accepted as being physiologically important satisfy the ciliate requirements. Nine amino acids are to be classed as indispensable for this strain (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, and valine) while arginine is synthesized at so low a rate that its inclusion becomes obligatory. Serine is extremely stimulatory, but its place may be taken by others of the dispensable amino acids (Kidder and Dewey, 1945c). The list of essential growth factors for this strain is not long. Purines (most effectively guanine) and pyrimidines (cytidylic acid and/or uracil) must be supplied in rather large amounts (Kidder and Dewey, 1945d), and folic acid must be present in amounts in excess of that required for most of the folic acid-requiring bacteria (Kidder, 1945). Factor II must be supplied. This substance (or substances) is still chemically undefined, but it possesses similarities to the "streptogenin" of Woolley (1941) and Sprince and Woolley (1944).

Biosynthesis of riboflavin, pantothen, and biotin can be accomplished by *T. geleii* W. Indefinitely transplantable growth results without exogenous thiamine (Kidder and Dewey, 1942; 1944; 1945b), riboflavin, pantothen, biotin, niacin, or pyridoxine. There is some evidence to indicate that *p*-aminobenzoic acid may not be essential, and the status of inositol and choline is still unknown.

For practical purposes it is always of advantage to include any substances of a stimulatory nature. The absence of any one of the stimulatory substances (thiamine, riboflavin, pantothen, biotin, niacin, pyridoxine) will become a limiting factor, decreasing the growth rate or the maximum yield or the longevity of the culture (Johnson and Baker, 1942; Hall, 1944). The stimulatory vitamins should be included in the culture medium of this ciliate when maximum growth is desired.

SUMMARY

1. It has been possible to prepare media for the growth of *Tetrahymena geleii* W which are free of riboflavin, pantothen, biotin, niacin, and pyridoxine, as determined by the *Lactobacillus casei* test.

2. *T. geleii* W is not dependent on an exogenous source of any one of the above vitamins. Omission of any one, however, reduces the maximum yield and, with the single exception of pantothen, the growth rate.

3. Biosynthesis of appreciable amounts of riboflavin, pantothen, and biotin occurs. These vitamins are found bound in the cell protoplasm. No significant increases of pyridoxine by biosynthesis were found.

4. The five vitamins listed are not essential growth factors for *T. geleii* W but are stimulatory factors, and as such should be included in the medium for optimum growth.

LITERATURE CITED

Cheldelin, V. H., M. A. Eppright, E. E. Snell, and B. M. Guirard, 1942. Enzymatic liberation of B vitamins from plant and animal tissues. *Univ. Texas Publ.* No. 4237: 15–36.

Eakin, R. E., E. E. Snell, and R. J. Williams, 1941. The concentration and assay of avidin, the injury producing protein in raw egg white. *Jour. Biol. Chem.*, **140**: 535–543.

Garnjobst, L., E. L. Tatum, and C. V. Taylor, 1943. Further studies on the nutritional requirements of Colpoda duodenaria. *Jour. Cell. Comp. Physiol.*, **21**: 199–212.

Hall, R. P., 1944. Comparative effects of certain vitamins on populations of Glaucoma piriformis. *Physiol. Zool.*, **17**: 200–209.

Hall, R. P., and W. B. Cosgrove, 1944. The question of the synthesis of thiamine by the ciliate, Glaucoma piriformis. *Biol. Bull.*, **86**: 31–40.

Hochberg, M., D. Melnick, L. Siegel, and B. L. Oser, 1943. Destruction of vitamin B_6 (pyridoxine) by light. *Jour. Biol. Chem.*, **148**: 253–254.

Hutchings, B. L., and W. H. Peterson, 1943. Amino acid requirement of Lactobacillus casei. *Proc. Soc. Exp. Biol. Med.*, **52**: 36–38.

Kidder, G. W., 1941. Growth studies on ciliates. V. The acceleration and inhibition of ciliate growth in biologically conditioned medium. *Physiol. Zool.*, **14**: 209–226.

Kidder, G. W., 1945. Studies on the biochemistry of Tetrahymena. VI. Folic acid as a growth factor for T. geleii W. *Arch Biochem.* (in press).

Kidder, G. W., and V. C. Dewey, 1942. The biosynthesis of thiamine by normally athiaminogenic microorganisms. *Growth*, **6**: 405–418.

Kidder, G. W., and V. C. Dewey, 1944. Thiamine and Tetrahymena. *Biol. Bull.*, **87**: 121–133.

Kidder, G. W., and V. C. Dewey, 1945a. Studies on the biochemistry of Tetrahymena. III. Strain differences. *Physiol. Zool.*, **18**: 136–157.

Kidder, G. W., and V. C. Dewey, 1945b. Studies on the biochemistry of Tetrahymena. IV. Amino acids and their relation to the biosynthesis of thiamine. *Biol. Bull.*, **89**: 131–143.

Kidder, G. W., and V. C. Dewey, 1945c. Studies on the biochemistry of Tetrahymena. I. Amino acid requirements. *Arch. Biochem.*, **6**: 425–432.

Kidder, G. W., and V. C. Dewey, 1945d. Studies on the biochemistry of Tetramymena. V. The chemical nature of Factors I and III. *Arch. Biochem.* (in press).

Kidder, G. W., C. A. Stuart, V. G. McGann, and V. C. Dewey, 1945. Antigenic relationships in the genus Tetrahymena. *Physiol. Zool.*, **18**: 415.

Landy, M., and D. M. Dicken, 1942. A microbiological assay method for six B vitamins using Lactobacillus casei and a medium of essentially known composition. *Jour. Lab. Clin. Med.*, **27**: 1086–1092.

Sprince, H., and D. W. Woolley, 1944. Relationship of a new growth factor required by certain hemolytic streptococci to growth phenomena in other bacteria. *Jour. Exp. Med.*, **80**: 213–217.

Stokes, J. L., M. Gunness, and J. W. Foster, 1944. Vitamin content of ingredients of microbiological culture media. *Jour. Bact.*, **47**: 293–299.

Tatum, E. L., and G. W. Beadle, 1942. The relation of genetics to growth factors and hormones. *Growth*, **6**: 27–35.

Tatum, E. L., L. Garnjobst, and C. V. Taylor, 1942. Vitamin requirements of Colpoda duodenaria. *Jour. Cell. Comp. Physiol.*, **20**: 211–224.

Wilson, D., R. C. Grauer, and E. Saier, 1940. A simplified continuous extractor for estrogens and androgens. *Jour. Lab. Clin. Med.*, **26**: 581–585.

Woolley, D. W., 1941. A new growth factor required by certain hemolytic streptococci. *Jour. Exp. Med.*, **73**: 487–492.

THE STRUCTURE OF MEIOTIC CHROMOSOMES IN THE GRASSHOPPER AND ITS BEARING ON THE NATURE OF "CHROMOMERES" AND "LAMP-BRUSH CHROMOSOMES"

HANS RIS

Rockefeller Institute for Medical Research, New York[1]

The nature of the gene is one of the fundamental problems in modern biology. Since the genes are located in the chromosomes, the structure, chemistry, and metabolism of the chromosomes are of special significance for the understanding of the gene and gene action. The prevalent interpretation of chromosome structure has developed as a kind of compromise between two originally opposed views, the "chromomere hypothesis" of Balbiani, Pfitzner, and Strasburger and the "chromonema hypothesis" of Baranetzky, Bonnevie, and Vejdovsky.[2] According to the "chromomere hypothesis," the chromosome consists of a series of small beads or discs strung together. During prophase they approach each other, fuse into larger complexes, and finally disappear in the thick rod-shaped metaphase chromosomes. For the "chromonema hypothesis" on the other hand, the fundamental unit of the chromosome is a coiled thread, tightly wound in a helix at metaphase and more or less uncoiled during interphase. Both chromomeres and spirals were discovered about the same time (Balibiani, 1876; Pfitzner, 1882; Baranetzky, 1880). Yet more and more structures first described as "chromomeres" have turned out to be coils and today the "chromomere" is in full retreat into the submicroscopic level. Strasburger's "chromomeres" in Tradescantia pollen mother cells had been clearly shown to be spirals by Baranetzky (1880); Pfitzner's "granules" in somatic prophases of the salamander were resolved into coils by Schneider (1910) and by Lee (1921), who concluded that all "chromomeres" are in reality turns in the helix. The modern view which is accepted by most cytologists today and is based mainly on Heitz (1935), holds that the true "chromomeres" (Belling's ultimate chromomeres) can only be seen in the prophase of meiosis (leptotene) and in the curious giant chromosomes of dipteran larvae, where the chromonemata are assumed to be completely uncoiled. According to this view (Reuter, 1930; Heitz, 1935; Darlington, 1937; White, 1937; Geitler, 1938; Koltzoff, 1938; Kuwada, 1939; Nebel, 1939; Huskins, 1941, 1942; Straub, 1943) the chromonema consists of chromomeres of different but constant size, rich in nucleic acid, connected by protein fibrils. The chromomeres bear the genes, they reproduce as specific units and they synapse in meiotic prophase. They are the visible expression of the linear arrangement of the genes.

---

[1] Part of the work for this paper was done in the Department of Biology, Johns Hopkins University.

[2] The "vacuolization hypothesis" of Grégoire and his school, denying both chromomeres and chromonemata, has been thoroughly disproved by the work of the last twenty years and need not be discussed here.

Yet even in leptotene chromosomes the "chromomeres" were found to be coils by several authors. They were first described as such in Tradescantia by Kaufmann (1931), who nevertheless accepted the "chromomere" interpretation for other plants and animals (Kaufmann, 1936). Koshy (1934, 1937) found the leptotene chromosome to be coiled in Allium and Aloe, Naithani (1937) in Hyacinthus. Smith (1932) suggested that the beadlike appearance of the leptotene in Galtonia might be due to twists in the chromonema and Hoare (1934) noted that the zygotene threads give the impression of two tightly coiled chromonemata. Kuwada (1939) pointed out that sharp turns in the coils might easily be mistaken for "chromomeres." In Tradescantia, Swanson (1943) found no "chromomeres" which could not be resolved into coils, and he suggested that a chromomere pattern such as that in maize might be due to differential spiralization.

Yet most recent discussions on the gene and chromosome structure cling tenaciously to the belief that "chromomeres" are real (e.g., Schultz, 1944). The main evidence usually presented, besides the salivary chromosomes of dipteran larvae, is the observations of Wenrich (1916), Lewis and Robertson (1916), and Chambers (1924) on the large chromosomes in grasshopper spermatocytes. To re-examine this evidence is the purpose of the present investigation.

Material and Methods

Spermatocytes of *Chorthippus curtipennis, Chorthophaga viridifasciata, Dissosteira carolina, Melanoplus femur-rubrum, Arphia* sp., *Hippiscus* sp., and *Orphulella* sp. were studied in sections (fixation: B 15 and Sanfelice, stain: Feulgen), and aceto-orcein smears. For the detailed study of leptotene chromosomes sections stained with Feulgen were found to be more reliable than smears. To uncoil chromosomes, testes were submersed for one-two hours in $2 \cdot 10^{-3}$ M KCN in Bělař solution (Bělař, 1929) before smearing (Oura, 1936). The optics used consisted of a Zeiss aplantic condenser N.A. 1.4, Zeiss 2 mm. objective N.A. 1.4 and 15× ocular. The photographs (except Figure 12) were taken with the same optics and a Bausch and Lomb photomicrographic camera type H. The stereoscopic photographs were made by shifting the substage diaphragm maximally to the left and right respectively for the two exposures.[3]

The Structure of Leptotene Chromosomes

On casual examination the slender, irregularly twisted chromosomes at leptotene have a beaded appearance as has been so often described in the literature (for a review see Reuter, 1930). A detailed study with the best optics and a delicate use of the fine adjustment screw of the microscope, however, resolves the beads or "chromomeres" into turns of a narrowly pitched coil[4] (Figures 1, 6a, and 13). With Feulgen the chromosome stains evenly throughout its length and there are no Feulgen-negative "interchromomeric fibrils." This uniform nature of the leptotene chromosomes can best be seen in well fixed sections. A chromosome, followed with the fine adjustment as it winds itself through the nucleus, is seen to be a thread of uniform thickness thrown into a tight, irregular helix. The narrow turns of this coil where the chromosome overlaps itself, appear as "chromomeres." The gyres can vary in width and may be unevenly spaced (see Figure 13). This can give the impression of different sized chromomeres. The width of the thread and the tightness of the helix are characteristic for each species of grasshopper studied. In aceto-orcein smears, when the chromosome has been under shear or pressure, an apparent chromomeric structure is more pronounced. This is due to the wax-like consistency of the chromosome which causes its gyres to fuse or be pulled out and otherwise distorted. Chromosomes, in which the coils can be clearly seen, can easily be transformed into the classical string of beads simply by exerting pressure on the coverslip and smearing them out. It is interesting to note in this connection that Belling (1931) emphasized that chromomeres are not clear in sections and that one has to use smears to make them visible.

---

[3] I wish to thank Mr. John Spurbeck, Dept. of Biology, Johns Hopkins University, for help with the photomicrographs.

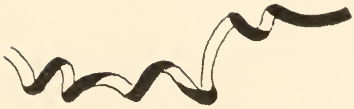
[4] Mr. L. Vanderlyn, Dept. of Zoology, University of Pennsylvania, informs me that he has come independently to the conclusion that the "chromomeres" are in reality gyres in the chromonemata. In a forthcoming paper he will trace the origin of these from the unpacking coils of the preleptotene in *Podisma alpina*.

When does that tight irregular coil of the leptotene chromosome originate? Is there any stage when the chromonemata are completely stretched out and without any signs of coiling? In all the grasshoppers studied no chromosome was found that did not show some degree of coiling. Furthermore, the characteristic coil of the leptotene chromosome is already present in the interphase and unravelling stage of preleptotene. We must assume that the leptotene spiral originates in the interphase or telophase of the preceding division. This origin of a prophase helix in the preceding telophase has been demonstrated by Sparrow (1942) in the microspore division in Tradescantia. The chromosome of the unravelling stage is thus doubly coiled (Figure 7). It shows the wide gyres of the previous metaphase relaxing into the relic coils of leptotene and the small tight helix which is destined to enlarge during pachytene and become the major coil of the first meiotic metaphase chromosome. This structure of the preleptotene chromosome was indicated clearly in McClung's figures for *Mecostethus lineatus* (esp. Figure 43, McClung, 1927). The heteropycnotic X chromosome in the prophase of grasshopper spermatocytes, which does not unwind in preleptotene and is thus comparable to the preleptotene autosomes in structure, similarly discloses a small tight helix and a wide irregular coil as Coleman (1943) has demonstrated.

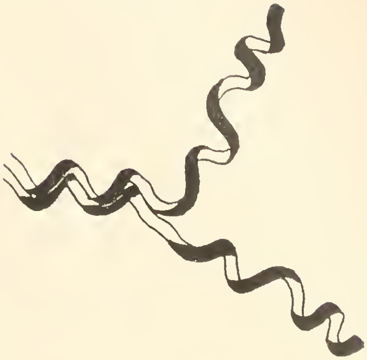
Since the preleptotene chromosome consists of at least two chromonemata the leptotene chromosome also must be double (Robertson, 1931). The split between the chromatids can sometimes be discerned, especially in the turns of the coil, but usually the sister strands are closely appressed. They seem to form a plectonemic spiral, though this could not be determined with certainty.

The Structure of Zygotene Chromosomes

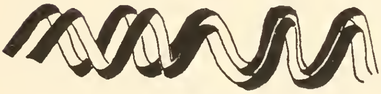
The pairing of homologous chromosomes at zygotene thus takes place between two coiled structures. The gyres of the two chromosomes fit into each other and become more or less closely appressed (Figures 2 and 6b). The bivalent now forms a paranemic coil. Just as the gyres in leptotene were mistaken for "chromomeres," so the gyres of the parallel coil in the bivalent were thought to be paired "chromomeres."



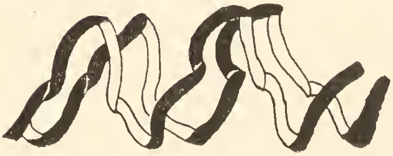
1



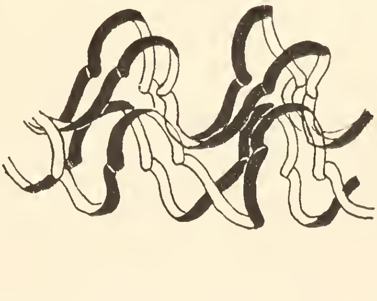
2



3



4



5

Figures 1–5. Diagrammatic representation of chromosome structure during meiotic prophase of the grasshopper.

Figure 1. Leptotene.

Figure 2. Zygotene.

Figure 3. Pachytene. The homologues can be either slightly separated or closely appressed.

Figure 4. Later pachytene. Appearance of the minor coil.

Figure 5. Diplotene. The chromonemata have separated laterally. This represents in essence also the structure of "lamp-brush chromosomes."

THE STRUCTURE OF PACHYTENE CHROMOSOMES

During pachytene the helices of the paired chromosomes increase in width and the number of gyres decreases. This process is identical to that described by Swanson (1942a) for Tradescantia (despiralization cycle). If the chromosomes are closely appressed only one helix is visible. When the coils separate slightly a reticular or vacuolated appearance is produced, though often two parallel helices can be clearly discerned (Figures 3 and 8). In late pachytene an irregular waviness appears on the gyres of the pachytene coil; this sometimes looks like a very fine spiral of narrow pitch. It most likely corresponds to the minor spiral described in plant chromosomes (Figures 4 and 9).

THE STRUCTURE OF THE CHROMOSOMES DURING DIPLOTENE AND DIAKINESIS

In this stage the chromosomes are most difficult to analyze. They are usually described in the literature as diffuse, having fuzzy or woolly fringes (see for instance Nebel and Ruttle, 1937). The better the general fixation seems to be, the less distinct or sharp the chromosomes appear. However, after submersing the cells for one to two hours in $2 \cdot 10^{-3}$ M KCN in Bělař solution and staining in aceto-orcein, the structure of the diakinesis chromosome and the reason for its woolly appearance becomes quite clear. The lateral separation of the chromonemata which had already begun in pachytene has progressed much further, so that their gyres now overlap only within a narrow central region. This region appears as a beaded darker core of the chromosome. The gyres of the major coil of the chromonemata form loops projecting beyond this central core (Figures 5 and 14). It is these loops of the individual chromonemata which give the chromosome its hairy appearance. If the separation of the coiled threads is great the chromosome looks like a dark, beaded rod with loops or hairs at regular intervals (Figure 14a). When the lateral shifting is less the chromosome gives the impression of a double beaded rod, the loops or hairs now of course being shorter (Figure 14b). These appearances can easily be explained on a model of four simultaneously coiled wires. Sometimes one or more irregular turns of the minor coil can be seen on the loops.

In this stage there is further evidence against the reality of "chromomeres." If the apparent thickenings in the leptotene chromosome were constant units of definite size, they should be visible also in the loops of the diplotene chromatids.

PLATE I

FIGURE 6. Chorthophaga, zygotene. Pretreated with ammonia vapor. Aceto-orcein smear. Note the coil of the univalent at *a* and the paranemic helix of the bivalent at *b*.

FIGURE 7. Chorthophaga, preleptotene. Aceto-orcein smear. Irregular "major coil" in the process of unravelling. The narrowly pitched helix ("minor coil") corresponds to the leptotene spiral (arrows).

FIGURE 8. Chorthippus, early pachytene. Section. Fixed with Sanfelice and stained with Feulgen.

FIGURE 9. Hippiscus, late pachytene. Section. Fixed with Sanfelice and stained with Feulgen.

FIGURES 10 AND 11. Orphulella, pachytene. Pretreated for 2 hours in KCN. Aceto-orcein smear. The heterochromatic knobs have been resolved into coils (arrows).

FIGURE 12. Fragment of a "lamp-brush chromosome" from a frog oöcyte. Aceto-orcein smear. Note the loops of the major coil and the minor coil (arrows). Zeiss 3 mm. objective, 15 × ocular.

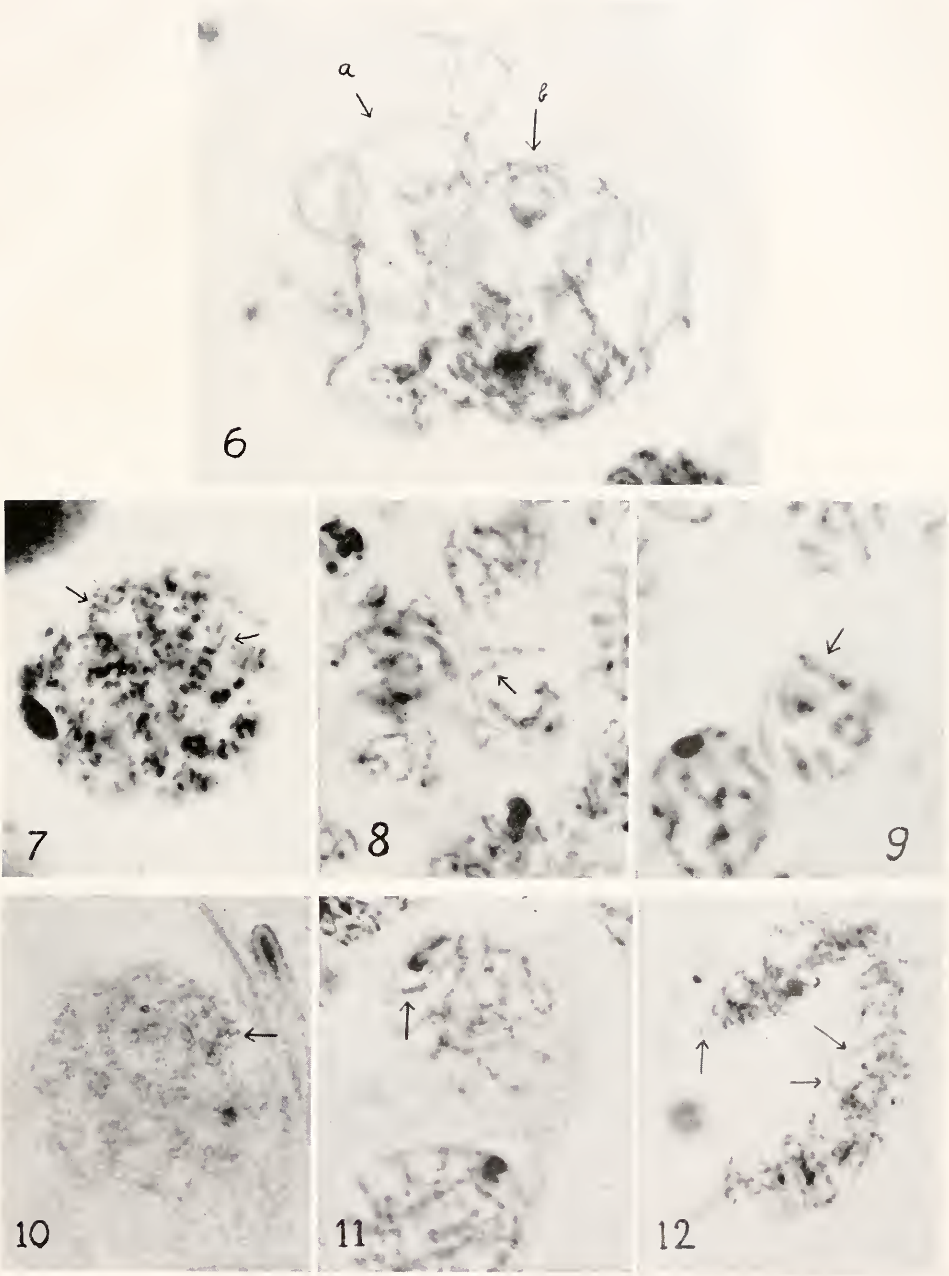

a
b
6
7
8
9
10
11
12

Plate I

These chromatids, however, never show any beaded structure. The despiralization already noted in pachytene has continued and has resulted in an increase in width and decrease in the number of gyres with a consequent shortening and thickening of the chromosome.

The Structure of Metaphase Chromosomes

At the end of diakinesis the gyres of the chromatids become more closely spaced along the chromosome axis, leading to a further shortening of the chromosome and a fusion of the "chromatic coating" (Ris, 1942) of the individual chromatids, so that a uniformly staining body results. The chromatids retain their lateral separation, causing what is sometimes observed as a reticulate or vacuolated appearance of the metaphase chromosomes.

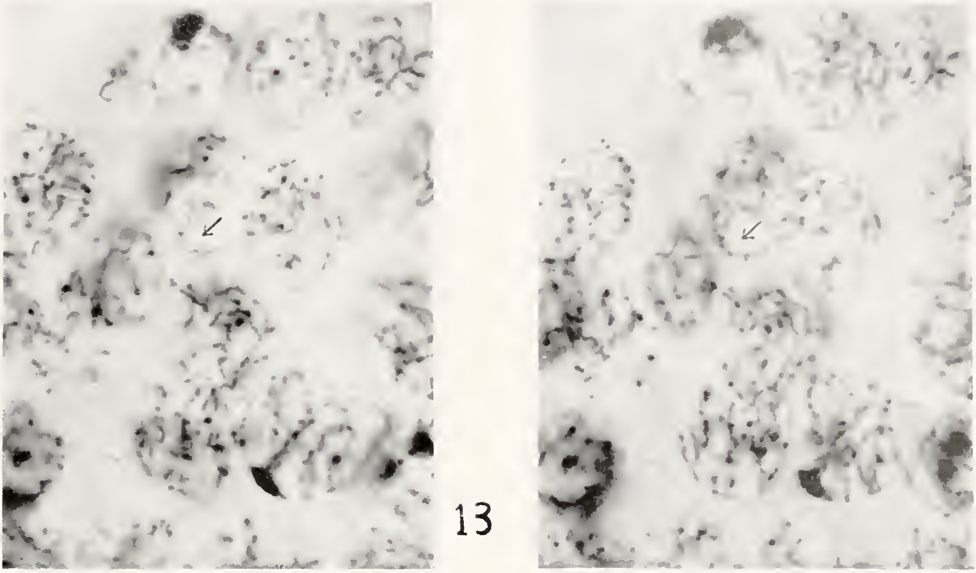
The Nature of the Heteropycnotic Regions in Orphulella

During meiotic prophase the chromosomes of Orphulella carry small, knob-like, darkly staining bodies, especially at their ends. These structures resemble the large "chromomeres" described by Wenrich (1916) in Phrynotettix. Treatment with KCN for 3 hours causes a loosening of the chromosome helix and shows that these knobs are tightly coiled regions of the chromosome (Figures 10 and 11). It is evident that the different appearance of such heteropycnotic regions in meiotic chromosomes is mainly due to differential coiling of the chromonemata as has been shown for the X chromosome by Coleman (1943). Similarly Wilson and Boothroyd (1944) have demonstrated that heterochromatic differentiations after cold treatment are the result of differential coiling.

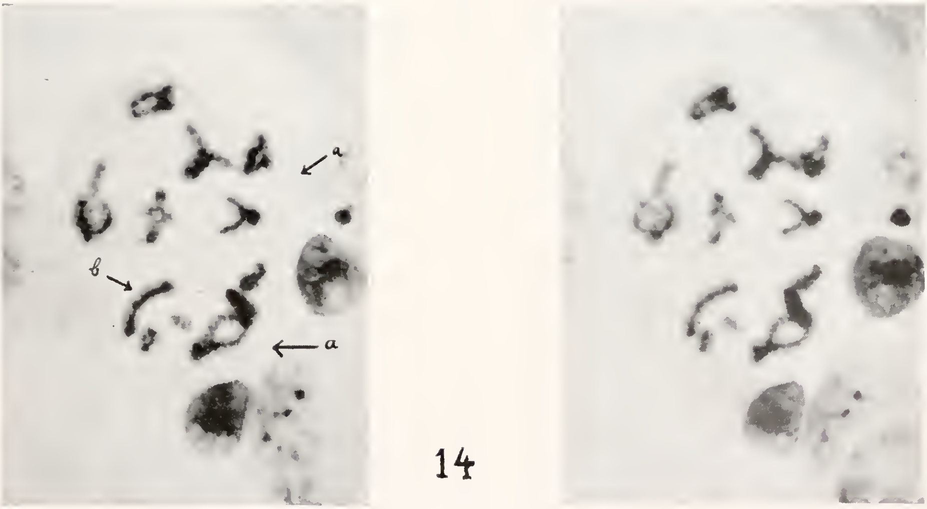
Discussion

Chromomeres

The synthesis of cytology and genetics in the chromosome theory of inheritance has had a stimulating effect on the investigation of chromosomes. Yet the knowledge of the intimate structure of the chromosome has been retarded rather than furthered by the influence of genetics. The constant desire to find visual expression of the linear order of genes has led to the perpetuation of misinterpretations of the microscopic image. Indeed cytogenetics has established beyond doubt the longitudinal differentiation of chromosomes, but it is not justifiable to conclude that the units of this differentiation are microscopically visible particles. Thus observations which did not agree with the "chromomere" hypothesis tended to be ignored. The extensive literature on the subject (see Reuter, 1930) shows the widespread acceptance as well as the great versatility of the chromomere concept. Almost any expression of unevenness along the chromosome was at one time or other called "chromomere." The first pictures of "chromomeres" were published by Balbiani (1876) and Pfitzner (1882). Both described prophase and metaphase chromosomes in somatic cells. Today there can be no doubt that they saw the gyres of the somatic helix (Schneider, 1910; Lee, 1921; Creighton, 1938). Strasburger (1882) and Farmer and Shove (1905) described disc-like "chromomeres" in meiotic metaphase chromosomes of Tradescantia. We know now that they mistook the gyres of the major coil for discs. Quite often chromocenters in



13



14

Plate II

Figure 13. Stereophotomicrograph, Chorthippus leptotene. Section. Fixed with Sanfelice and stained with Feulgen and Iron hematoxylin. Note the coiled leptotene chromosomes (arrow).

Figure 14. Stereophotomicrograph, Hippiscus diakinesis. Pretreated with KCN. Aceto-orcein smear. Note the loops of the major coil which give the chromosomes at this stage the fuzzy appearance.

interphase nuclei and heteropycnotic regions on the chromosome, such as found in the X chromosome of *Notonecta indica* (Browne, 1916), were called "chromomeres" (cf. Heitz, 1929). Shinke (1937) and Coleman (1940, 1941) have shown that such heteropycnotic regions are parts of the chromonema which remain tightly coiled or become precociously coiled. This could be confirmed in the present paper for the "knobs" of the meiotic chromosomes of Orphulella. Thus, one more "chromomere" was reduced to chromonematic coiling. There remained the "ultimate chromomere" of Belling (1928), the only bona fide "chromomere" according to most modern cytologists. This "chromomere" can only be seen in meiotic prophase and in salivary chromosomes of dipteran larvae, where the chromonemata are assumed to be maximally stretched. Let us examine point for point the evidence which is given for the reality of these "chromomeres" (see reviews cited in introduction).

(*a*) *"The chromomeres are seen in living cells and cannot be artefacts."* Bělař (1928) described "chromomeres" in living spermatocytes of the grasshopper. An analysis of his figure shows that he did not see chromomeres but the coils of diakinesis chromosomes. Lewis and Robertson (1916) and Chambers (1924) found "chromomeres" in the leptotene of living grasshopper spermatocytes. This may show that the structures observed are not fixation artefacts, but it certainly is easier to misinterpret narrow coils as granules in unstained cells where the chromosomes are hardly visible, than in well stained preparations. Yet there is a very interesting observation by Chambers (1924, page 270) which seems to have been overlooked by himself as well as most reviewers of chromosome structure. He writes: "If one of the early prophase chromosomes with ragged granular outlines be seized with a needle and rapidly pulled across the field so as to stretch it, the granules disappear and the whole substance becomes homogeneous." So Chamber's microdissection study does not support the "chromomere" hypothesis, but rather the assumption of a uniform but coiled leptotene chromosome.

(*b*) *"The chromomeres have specific and constant sizes and form a definite pattern."* The classical examples are Dendrocoelum (Gelei, 1921) and Phrynotettix (Wenrich, 1916). The observed patterns in these and other forms are an expression of the longitudinal differentiation of the chromosome. This differentiation is real. But the nature of this differentiation now turns out to be differential coiling and not a sequence of discrete bodies of different sizes. The large "chromomeres" in Phrynotettix are heterochromatic regions along the chromosome similar to those found in certain plant chromosomes and those described for Orphulella in this paper. In *Veltheimia viridifolia* Coleman (1940) could show that such heterochromatic regions are closely coiled sections of the chromonema. They correspond in structure to the differential segment in Rhoeo (Coleman, 1941) and the chromocenters in various animals and plants (Shinke, 1937). The knobs in maize are most probably of a similar nature.

(*c*) *"The chromomeres of homologous chromosomes pair specifically at zygotene."* Just as the turns in the spiral give the impression of "chromomeres" at leptotene, the paranemic spiral of the paired bivalent simulates a row of paired granules. Since homologous regions of the chromosomes pair, it is evident that heterochromatic sections will come to lie side by side in the pachytene chromosomes.

(*d*) *"The number of chromomeres in leptotene corresponds approximately to the number of genes in Lilium* (Belling, 1928). *In salivary chromosomes the* *bands, which correspond to the leptotene chromomeres, were shown to be closely associated with certain genes* (Muller and Prokofyeva, 1935)."

Belling's estimate of the number of genes in Lilium was entirely arbitrary and he had no direct evidence for a correlation of "ultimate chromomeres" and genes. In salivary chromosomes of Drosophila, however, a great number of workers have proven beyond doubt that the visible "bands" are correlated with certain genes. A recent analysis of the salivary chromosomes of Sciara in collaboration with Dr. Helen Crouse (in press) has shown that the "granules" and "bands" are misinterpretations of a very complicated spiralization of a bundle of chromonemata. What has been described as a "chromomere" corresponding to a gene represents in reality a region of relatively considerable length along the chromonema. The cytogenetic work on Drosophila salivary chromosomes is not evidence for a "chromomeric" structure of the chromonema, but shows that certain sections of the uniform chromonematic thread correspond to definite genes and that the detailed nature of the coiling in these interphase chromosomes is closely correlated with a genetic specificity on a submicroscopic level.

In summary this is the evidence against the existence of "chromomeres": (a) In living cells the microdissection experiment of Chambers (1924) shows that the leptotene chromosome can be stretched into a uniform thread. (b) In several plants such as Tradescantia (Kaufmann, 1931; Swanson, 1943), Allium and Aloe (Koshy, 1934, 1937), Hyacinthus (Naithani, 1937), and in the grasshopper the leptotene chromosome consists of a uniform, coiled thread, Feulgen-positive throughout its length. No evidence of interchromomeric fibrils can be found. The leptotene coils can be followed into the pachytene where they increase in width and decrease in number. This explains the observation of many authors (e.g., Belling, 1931) that the "chromomeres" increase in size and decrease in number during the course of prophase. (c) In the diplotene chromosomes of the grasshopper no "chromomeres" can be seen in the large loops of the chromatids. If specific "chromomeric" granules were present at leptotene they should be visible also in the chromonema of diplotene. (d) McClintock (1944) has shown in maize that at least one gene is located in the interchromomeric thread between the terminal knob and the first "chromomere" on chromosome nine. This disproves definitely the idea, at least for maize, that the genes are necessarily located in the "chromomeres" which are connected by non-genic fibrils.

Diplotene chromosomes and "lamp-brush chromosomes"

The coiling cycle in the grasshopper appears to be identical with that described by Swanson (1942, 1943) for Tradescantia. The leptotene coil develops into the major coil of diakinesis and metaphase through despiralization. There is no definite minor coil, but from late pachytene on, an irregular waviness appears on the loops of the chromatids, resembling an incipient helix. A minor coil was seen in spermatocytes of another orthopteran, Podisma, by Makino (1936). In Trillium (Huskins, 1941) there seems to be a similar waviness instead of a definite helix as was demonstrated for Tradescantia. This difference in the appearance of the minor coil seems to be mainly one of timing of the spiralization cycle as Kuwada (1938) has suggested. In the grasshopper the chromatids have never been seen completely separated in diakinesis or metaphase. Their coils sometimes appear interlocked as Kuwada (1938) found in Tradescantia, but this could not be definitely determined. Swanson (1942b) has shown that the terminalization of chiasmata is correlated with the despiralization of the major coil in Tradescantia. The same process takes place in the grasshopper and it is most likely that here, too, terminalization of chiasmata is the consequence of despiralization of the major coil.

The diffuse appearance of orthopteran as well as most other animal chromosomes in diplotene has made their analysis rather difficult. The chromonema is generally of smaller diameter than in plant chromosomes and therefore the delicate loops of the major coils escaped observation. This diffuse structure is due to a lateral separation of the chromatids in contrast to the usual appression of the chromatids in plant chromosomes. Under certain conditions, and especially in diakinesis, plant chromosomes also show a separation of chromatids. They then give the same pictures as diplotene chromosomes of animals (see the anaphase chromosome of desynaptic Trillium in Figure 9 of Sparrow, Huskins and Wilson, 1941; Swanson, 1942a, 1943, and Kuwada and Nakamura, 1938 for Tradescantia). Plant and animal chromosomes have often been described as reticulate or vacuolated. Grégoire and his school based on this their "vacuolization hypothesis" of chromosome structure. All their pictures can today be explained on the simple assumption of a multiple stranded helix with the chromonemata more or less appressed or opened up.

When the lateral separation of the chromonemata is great and the loops only faintly stained, the chromosome may appear covered with a layer of achromatic material (often described as "matrix" or "sheath"; see for instance Lee, 1921 and McClung, 1941, Figure 7). Probably many a "matrix" in the literature is nothing but the apparant connection between faintly staining outer loops, running at an even distance from the darker core of the chromosome where the chromonemata overlap. Makino (1936) published some photographs of diakinesis and metaphase chromosomes of Podisma which at first seem to contradict my interpretation of these stages. He shows a dark inner coil sometimes appearing double, surrounded by a light "matrix." Faint strands are sometimes seen to connect the central spiral with the border of the "matrix." Yet it is very easy to understand these figures with the help of a model of four wires coiled together. When two are maximally separated laterally and two stay appressed in the center, Makino's coil and matrix become explainable. The outer coils are not at all or only faintly stained in his gentian violet preparations and their outer boundary suggests the presence of a "matrix."

The previous studies of diplotene chromosomes of Orthoptera have completely ignored these outer gyres of the chromonemata. They were described as woolly threads or brushlike projections on the surface of the chromosome, but not as an essential part of it. Thus the pictures of Hearne and Huskins (1934), Nebel and Ruttle (1937), Darlington (1936), and the McClung school are based on optical illusions or too light staining. What were described as "chromomeres" in this stage are the points of overlap of the chromonemata. Darlington (1936) has studied relational coiling of chromatids and chromosomes in pachytene and diplotene. What he pictured as one single chromatid, however, is not a continuous structure, but a series of nodes of separate overlapping major coils. His relational coil of chromatids is therefore an optical illusion. Only a complete stretching of the major coil could reveal whether the chromatids are wound around each other (see Kuwada, 1938).

Many oöcytes and spermatocytes in diplotene undergo a so-called "diffuse stage," which is correlated with the growth of the cell. The chromosomes stain only faintly and lose their definite shapes; they may even disappear into a reticular structure. In the grasshopper the diffuse nature of the chromosomes is due to the loosening and separation of the individual chromonemata of the major coil. This more or less pronounced loosening up of the gyres, combined possibly with some chemical changes in the composition of the chromatin, can explain the appearance of diplotene chromosomes during this stage in spermatocytes and oöcytes.

The diplotene chromosomes in the large oöcytes of some vertebrates have particularly interested the cytologist ever since their discovery by Rückert in 1892, because of their tremendous size. Their fuzzy and brush-like appearance warranted the name "lamp-brush chromosomes." Duryee (1937, 1938, 1939, 1941) has recently studied these chromosomes in great detail in the frog and salamander, and concludes that (1) they represent paired gelatinous cylinders in which the chromomeres are embedded. (2) From these chromomeres lateral loops grow out. He likens this growth to that of a crystal or the reproduction of a virus. (3) In a later stage, before the maturation divisions take place, these lateral loops are thrown off into the cytoplasm as genic products essential for the early embryo.

Painter (1940) came to somewhat different conclusions. He considers "lamp-brush chromosomes" to be chromosome aggregates, which originated through endomitosis and the loops to correspond to whole chromosomes. Material from thousands of such chromosomes, he maintains, is thrown into the cytoplasm as substrate for the synthesis of cleavage chromosomes. Koltzoff (1938) thinks that the lateral projections are side branches of the chromomeres which then are given off into the cytoplasm.

In contrast to Duryee, Koltzoff, and Painter, it is here suggested that "lamp-brush chromosomes" are typical diplotene chromosomes which differ from other diplotene chromosomes only in the tremendous longitudinal growth of the chromonemata. The loops are then the major coils of the laterally separated chromonemata, the "chromomeres" are simply overlaps of the strands just as in diplotene chromosomes of the grasshopper. Figure 12 shows a fragment of a "lamp-brush chromosome" of a frog oöcyte, smeared in aceto-orcein. The somewhat distorted large loops of the major coil and the minor coil are easily visible.

The evidence for this interpretation may be summarized as follows: (a) The loops are continuous as Rückert (1892) has already observed. He followed the chromonema for several turns. He also pointed out that the granules ("chromomeres") are not real, but optical sections of the overlapping threads. The denser inner region of the chromosome he described as due to the radial arrangement of the threads. (b) "Lamp-brush chromosomes" are diplotene chromosomes and except for their greater size have the same appearance as the diplotene chromosome of the grasshopper. Since it has been shown here that the loops are simply the gyres of the major coil of the separate chromonemata, one can conclude that the corresponding appearance of the "lamp-brush chromosome" is the result of a similar structure. (c) Koltzoff (1938) has published drawings of cross sections of "lamp-brush chromosomes" (his Figure 10, b and c). These cross sections look like a star with characteristically eight rays. These eight rays are most likely the eight half-chromatids which form independent loops, though Koltzoff saw them as brush-like projections.

The reduction in chromosome size just before the meiotic divisions is accomplished then not by throwing off parts of the chromosome or entire chromosomes, but by elimination of material on a submicroscopic level.

The microscopic organization of chromosomes

Kuwada (1939) in his review of chromosome structure predicted that the spiral theory might well prove capable of harmonizing the various hypotheses of chromosome structure. Such a uniform interpretation of the structure of all types of chromosomes is now possible. The unit of the chromosome is the chromonema, a microscopically uniform thread. This chromonema is never completely straightened out, but always shows some degree of spiralization. This coiling is not at random, but, as the salivary chromosomes and heterochromatic regions show, is an expression of the longitudinal differentiation of the chromonema and closely correlated with the genes. It is, in other words, an expression of submicroscopic structure and possibly the functional state of the gene (cf. heterochromatin). The microscopic uniformity of course does not exclude a great variability of submicroscopic structure and chemical composition along the chromonema. During the mitotic cycle there develops a condensed chromosome through despiralization of the incipient coil of early prophase. The differentiation of mitotic chromosomes, primary and secondary constrictions, satellites, and heterochromatic regions are expressions of the differential coiling of the chromonemata. In the resting nucleus of different tissues we often find different patterns of heterochromatin. It may be that differential spiralization of the chromonemata in resting cells is correlated with cell differentiation. The chromonema is not uniform in length, but it can vary greatly from cell to cell in the same organism, as well as in the same cell in different metabolic states. In many synthetically very active cells as for instance some oöcytes, nurse cells, gland cells (dipteran salivary glands), the total amount of chromatin is greatly increased. This is accomplished by an increase in the number of chromosomes (endomitosis, cf. Geitler, 1941), by a growth in length of the chromonemata (as in "lamp-brush chromosomes") or by both simultaneously (salivary chromosomes). In "lamp-brush" and salivary chromosomes the increase in length is tremendous and would be difficult to understand if only inert "genoplasm" or "matrix" (Koltzoff, 1938) had increased. More likely it is an increase in the volume of the gene complex, related to the greater metabolic activity. We have to look at the gene, therefore, not as a unit of constant and specific size as expressed in the "chromomere" hypothesis, but as a complex that is greatly variable in mass, depending on the metabolic activity of the nucleus.

SUMMARY

1. "Chromomeres" do not exist as definite structures. What has been described as "chromomeres" are (a) misinterpretations of gyres of the chromonematic helix (leptotene, somatic prophase); (b) points of overlap of chromonemata (diplotene); (c) heterochromatic sections consisting of more tightly coiled regions of the chromonema. The fundamental unit of the chromosome is a microscopically

uniform thread. The longitudinal differentiation of the chromosome is due to differential coiling of this chromonema.

2. "Lamp-brush chromosomes" are typical diplotene chromosomes, but with tremendously enlongated chromonemata. The side branches are the gyres of the major coils of the individual chromonemata, which have laterally separated from each other.

I am greatly indebted to Prof. F. Schrader and Dr. S. Hughes-Schrader, Columbia University, for critically reading the manuscript.

LITERATURE CITED

Balbiani, E. G., 1876. Sur les phénomènes de la division du noyau cellulaire. *C. R. Acad. Sci. (Paris)*, **83**: 831–834.

Baranetzky, J., 1880. Die Kernteilung in den Pollenmutterzellen einiger Tradescantien. *Bot. Zeitg.*, **38**: 241–248, 265–274, 281–296.

Bělař, K., 1928. Ueber die Naturtreue des fixierten Präparates. *Verh. V. Int. Kongr. Vererbgswiss.*, **1**: 402–407.

Bělař, K., 1929. Beiträge zur Kausalanalyse der Mitose II. *Roux Arch., f. Entwick mech.*, **118**: 359–484.

Belling, J., 1928. The ultimate chromomeres of Lilium and Aloe with regard to the number of genes. *Univ. of Cal. Publ. in Bot.*, **14**: 307–318.

Belling, J., 1931. Chromomeres of liliaceous plants. *Univ. of Cal. Publ. in Bot.*, **16**: 153–170.

Browne, E. N., 1916. A comparative study of the chromosomes of six species of Notonecta. *Jour. Morph.*, **27**: 119–162.

Chambers, R., 1924. The physical structure of protoplasm as determined by micro-dissection and injection. *Cowdry's General Cytology*, 237–309.

Coleman, L. C., 1940. The cytology of Veltheimia viridifolia Jacq. *Amer. Jour. Bot.*, **27**: 887–895.

Coleman, L. C., 1941. The relation of chromocenters to the differential segments in Rhoeo discolor Hance. *Amer. Jour. Bot.*, **28**: 742–747.

Coleman, L. C., 1943. Chromosome structure in the Acrididae with special reference to the X chromosome. *Genetics*, **28**: 2–8.

Creighton, M., 1938. Chromosome structure in Amblystoma punctatum. *Cytologia*, **8**: 497–504.

Darlington, C. D., 1936. Crossing-over and its mechanical relationships in Chorthippus and Stauroderus. *Jour. Gen.*, **33**: 465–500.

Darlington, C. D., 1937. Recent Advances in Cytology. Blakiston.

Duryee, W. R., 1937. Isolation of nuclei and non-mitotic chromosome pairs from frog eggs. *Arch. exp. Zellf.*, **19**: 171–176.

Duryee, W. R., 1938. A microdissection study of amphibian chromosomes. *Biol. Bull.*, **75**: 345.

Duryee, W. R., 1939. Comparative effects of X-radiation on isolated and non-isolated nuclei. *Anat. Rec.*, **75** (Suppl.): 144.

Duryee, W. R., 1941. The chromosomes of the amphibian nucleus. *Univ. of Penn. bicent. Conf. Cyt. Genet. Evol.*, 129–141.

Farmer, J. B., and D. Shove, 1905. On the structure and development of the somatic and heterotype chromosomes of Tradescantia Virginica. *Quart. J. Micr. Sci.*, **48**: 559–570.

Geitler, L., 1938. Chromosomenbau, Protoplasma Monographien, **14**, Berlin.

Geitler, L., 1941. Des Wachstum de Zellkerns in tierischen und pflanzlichen Geweben. *Erg. d. Biol.*, **18**: 1–54.

Gelei, J., 1921. Weitere Studien über die Oogenese des Dendrocoelum lacteum II. Die Längskonjugation der Chromosomen. *Arch. f. Zellf.*, **16**: 88–169.

Hearne, E. M., and C. L. Huskins, 1934. Chromosome pairing in Melanoplus femur-rubrum. *Cytologia*, **6**: 123–147.

Heitz, E., 1929. Heterochromatin, Chromocentren, Chromomeren. *Ber. Dtsche Bot. Ges.*, **47**: 274–284.

Heitz, E., 1935. Chromosomenstruktur und Gene. *Z. ind. Abst. Ver.*, **70**: 402–447.

Hoare, G., 1934. A comparative study of the chromosomes of Scilla nonscripta during somatic and meiotic mitosis. *Cellule*, **43**: 5–42.

Huskins, C. L., 1941. The coiling of chromonemata. *Cold Spring Harbor Symp. on Quant. Biol.*, **9**: 13–17.

Huskins, C. L., 1942. Structural differentiation of the nucleus. The structure of protoplasm, ed. Seifriz, 109–126.

Kaufmann, B. P., 1931. Chromonemata in somatic and meiotic mitoses. *Amer. Nat.*, **65**: 280–282.

Kaufmann, H. P., 1936. Chromosome structure in relation to the chromosome cycle. *Bot. Rev.*, **2**: 529–553.

Koltzoff, N. K., 1938. The structure of the chromosomes and their participation in cell metabolism. *Biol. Zhurn.*, **7**: 3–46.

Koshy, T. K., 1934. Chromosome studies in Allium. II. The meiotic chromosomes. *Jour. Roy. Micr. Soc.*, **54**: 104–120.

Koshy, T. K., 1937. Number and behavior of chromosomes in Aloe litoralis. *Ann. of Bot., N. S.*, **1**: 43–58.

Kuwada, Y., 1938. Behavior of chromonemata in mitosis. VII. A chromosome study by the artificial uncoiling method of the chromonema spirales. *Cytologia*, **9**: 17–22.

Kuwada, Y., 1939. Chromosome structure. A critical review. *Cytologia*, **10**: 213–256.

Kuwada, Y., and T. Nakamura, 1938. Behavior of chromonemata in mitosis. VIII. The major spirals in diakinesis. *Cytologia*, **9**: 28–34.

Lee, A. B., 1921. The structure of certain chromosomes and the mechanism of their division. *Quart. Jour. Micr. Sci.*, **65**: 1–32.

Lewis, M. R., and W. R. B. Robertson, 1916. The mitochondria and other structures observed by the tissue culture method in the male germ cells for Chorthippus curtipennis Scudd. *Biol. Bull.*, **30**: 99–114.

McClintock, B., 1944. The relation of homozygous deficiencies to mutations and allelic series in maize. *Genetics*, **29**: 478–502.

McClung, C. E., 1927. Synapsis and related phenomena in Mecostethus and Leptysma (Orthoptera). *Jour. Morph.*, **43**: 181–265.

McClung, C. E., 1941. The tetramite of orthopteran spermatocytes. *Jour. Morph.*, **69**: 575–584.

Makino, S., 1936. The spiral structure of chromosomes in the meiotic divisions of Podisma (Orthoptera). *J. Fac. Sci. Hokkaido Imp. Univ.*, **5**: 29–40.

Muller, H., and A. A. Prokofyeva, 1935. The individual gene in relation to the chromomere and the chromosome. *Proc. Nat. Acad. Sci.*, **21**: 16–26.

Naithani, S. P., 1937. Chromosome studies in Hyacinthus orientalis L. II. The meiotic chromosomes. *Ann. Bot. N. S.*, **1**: 257–276.

Nebel, B. R., 1939. Chromosome structure. *Bot. Rev.*, **5**: 563–626.

Nebel, B. R., and M. L. Ruttle, 1937. Chromosome structure. XIII. Meiosis in Dissosteira carolina L. *Zeitschr. Zellf.*, **26**: 281–292.

Oura, G., 1936. A new method of unravelling the chromonema spirals. *Z. wiss. Mikr.*, **53**: 36–37.

Painter, T. S., 1940. On the synthesis of cleavage chromosomes. *Proc. Nat. Acad. Sci.*, **26**: 95–100.

Pfitzner, W., 1882. Ueber den feineren Bau der bei der Zellteilung auftretenden fadenförmigen Differenzierungen des Zellkerns. *Morph. Jahrb.*, **7**: 289–311.

Reuter, E., 1930. Beiträge zu einer einheitlichen Auffassung gewisser Chromosomenfragen. *Acta Zool. Fennica*, **9**: 1–487.

Ris, H., 1942. A cytological and experimental analysis of the meiotic behavior of the univalent X chromosome in the bearberry aphid Tamalia (= Phyllaphis) coweni (Ckll). *Jour. Exp. Zool.*, **90**: 267–330.

Robertson, W. R. B., 1931. Chromosome studies. II. Synapsis in the Tettigidae with special reference to the pre-synapsis split. *Jour. Morph.*, **51**: 119–146.

Rückert, J., 1892. Zur Entwicklungsgeschichte des Ovarialeies bei Selachiern. *Anat. Anz.*, **7**: 107–158.

Schneider, K. C., 1910. Histologische Mitteilungen III. Chromosomengenese. *Festschrift f. R. Hertwig*, **1**: 213–232.

Schultz, J., 1944. The gene as a chemical unit. In Alexander, Colloid-chemistry, vol. V, 819–850.

Shinke, N., 1937. An experimental study on the structure of living nuclei in the resting stage. *Cytologia, Fujii Jub.*, 449–463.

Smith, F. H., 1932. The structure of the somatic and meiotic chromosomes of Galtonia candicans. *Cellule*, **41**: 241–263.

Sparrow, A. H., 1942. The structure and development of the chromosome spirals in microspores of Trillium. *Can. Jour. Res.*, C **20**: 257–266.

Sparrow, A. H., C. L. Huskins, and G. B. Wilson, 1941. Studies on the chromosome spiralization cycle in Trillium. *Can. Jour. Res.*, C **19**: 323–350.

Strasburger, E., 1882. Ueber den Teilungsvorgang der Zellkerne und das Verhältnis der Kernteilung zur Zellteilung. *Arch. Mikr. Anat.*, **21**: 476–590.

Straub, J., 1943. Chromosomenstruktur. *Naturwiss.*, **31**: 97–108.

Swanson, C. P., 1942a. Meiotic coiling in Tradescantia. *Bot. Gaz.*, **103**: 457–474.

Swanson, C. P., 1942b. Some consideration on the phenomenon of chiasma terminalization. *Amer. Nat.*, **76**: 593–610.

Swanson, C. P., 1943. The behavior of meiotic prophase chromosomes as revealed through the use of high temperatures. *Amer. Jour. Bot.*, **30**: 422–428.

Wenrich, D. H., 1916. The spermatogenesis of Phrynotettix magnus with special reference to synapsis and the individuality of the chromosomes. *Bull. Mus. Comp. Zool. Harv. Univ.*, **60**: 57–135.

White, M. J. D., 1937. The Chromosomes. Methuen, London.

Wilson, G. B., and E. R. Boothroyd, 1944. Temperature-induced differential contraction in the somatic chromosomes of Trillium erectum L. *Can. Jour. Res.*, **22**: 105–119.

INDEX

ABSTRACTS of scientific papers presented at the Marine Biological Laboratory, Summer of 1945, 184.
Agglutination of starfish sperm by fertilizin, 84.
Annual report of the Marine Biological Laboratory, 1.
Arbacia punctulata, dilution medium and survival of the spermatozoa, 162.
Arbacia punctulata egg when centrifuged in single salt solutions, stratification and breaking of the, 72.
Azide and cyanide, the effect of, on the development of frogs' eggs, 122.

BIOCHEMISTRY of Tetrahymena, IV, 131.
Biochemistry of Tetrahymena, VII, 229.
Bryozoa, studies on fresh-water. XVI. Fredericella australiensis var. browni, n. var., 215.
Bryozoa, studies on marine. I. Aeverrillia setigera (Hincks) 1887, 201.

CHEMICAL factors influencing artificial activation of Nereis eggs, 144.
Ciliates from Phytia setifer, 95.
Cochliophilus depressus gen. nov., sp. nov. and Cochliophilus minor sp. nov., holotrichous ciliates from the mantle cavity of Phytia setifer (Cooper), 95.
Cyanide and azide, the effects of, on the development of frogs' eggs, 122.
Cyanide, effect of, on respiration of Paramecium caudatum and Paramecium aurelia, 76.

DEVELOPMENT of marine fouling communities, 103.
DEWEY, VIRGINIA C. *See* GEORGE W. KIDDER
Dilution medium and survival of the spermatozoa of Arbacia punctulata. I. Effect of the medium on fertilizing power, 162.
Dominant lethality and correlated chromosome effects in Habrobracon eggs x-rayed in diplotene and in late metaphase I, 61.

EFFECT of cyanide on respiration in Paramecium caudatum and Paramecium aurelia, 76.

FRESH-WATER Bryozoa, XVI, 215.
Frogs' eggs, the effects of cyanide and azide on the development of, 122.

GOLGI apparatus, in chicken gizzard epithelium by means of the quartz microscope, a study of the, 157.
Grasshopper, structure of meiotic chromosomes in the, 242.

HABROBRACON eggs x-rayed in diplotene and in late metaphase I, dominant lethality and correlated chromosome effects in, 61.
HARVEY, ETHEL BROWNE. Stratification and breaking of the Arbacia punctulata egg when centrifuged in single salt solutions, 72.
HAYASHI, TERU. Dilution medium and survival of the spermatozoa of Arbacia punctulata. I. Effect of the medium on fertilizing power, 162.
Heteroagglutinins in lobster-blood, 193.
Heterocineta phoronopsidis sp. nov., a ciliate from the tentacles of Phoronopsis viridis Hilton, 180.
HIBBARD, HOPE AND GEORGE I. LAVIN. A study of the Golgi apparatus in chicken gizzard epithelium by means of the quartz microscope, 157.
Holotrichous ciliates from the mantle cavity of Phytia setifer (Cooper), 95.

KIDDER, GEORGE W. AND VIRGINIA C. DEWEY. Studies on the biochemistry of Tetrahymena. IV. Amino acids and their relation to the biosynthesis of thiamine, 131.
KIDDER, GEORGE W. AND VIRGINIA C. DEWEY. Studies on the biochemistry of Tetrahymena. VII. Riboflavin, pantothen, biotin, niacin, and pyridoxine in the growth of T. geleii W, 229.
KOZLOFF, EUGENE N. Cochliophilus depressus gen. nov., sp. nov. and Cochliophilus minor sp. nov., holotrichous ciliates from the mantle cavity of Phytia setifer (Cooper), 95.
KOZLOFF, EUGENE N. Heterocineta phoronopsidis sp. nov., a ciliate from the tentacles of Phoronopsis viridis Hilton, 180.

LAVIN, GEORGE I. *See* HOPE HIBBARD, 157.
LEFEVRE, PAUL G. Certain chemical factors influencing artificial activation of Nereis eggs, 144.

MARINE Biological Laboratory, Annual report of the, 1.
Marine Bryozoa, I, 201.
Marine fouling communities, the development of, 103.
Meiotic chromosomes in the grasshopper, 242.
METZ, CHARLES B. The agglutination of starfish sperm by fertilizin, 84.
MOOG, FLORENCE. *See* S. SPIEGELMAN, 122.

NEREIS eggs, certain chemical factors influencing artificial activation of, 144.

PACE, D. M. The effect of cyanide on respiration in Paramecium caudatum and Paramecium aurelia, 76.
Paramecium caudatum and Paramecium aurelia, effect of cyanide on respiration of, 76.

RIS, HANS. The structure of meiotic chromosomes in the grasshopper and its bearing on the nature of "chromomeres" and "lamp-brush chromosomes," 242.
ROGICK, MARY DORA. Studies on fresh-water Bryozoa. XVI. Fredericella australiensis var. browni, n. var., 215.
ROGICK, MARY DORA. Studies on marine Bryozoa. I. Aeverrillia setigera (Hincks) 1887, 201.

SCHEER, BRADLEY T. The development of marine fouling communities, 103.
SCHEER, BRADLEY T. *See* ALBERT TYLER, 193.
Scientific papers presented at the Marine Biological Laboratory, Summer of 1945, 184.
SPIEGELMAN, SOL AND FLORENCE MOOG. A comparison of the effects of cyanide and azide on the development of frogs' eggs, 122.
Starfish sperm, agglutination of, by fertilizin, 84.
Stratification and breaking of the Arbacia punctulata egg when centrifuged in single salt solutions, 72.
Structure of meiotic chromosomes in the grasshopper and its bearing on the nature of "chromomeres" and "lamp-brush chromosomes," 242.
Studies on the biochemistry of Tetrahymena. IV. Amino acids and their relation to the biosynthesis of thiamine, 131.

TETRAHYMENA, studies on the biochemistry of, 131.
TYLER, ALBERT AND BRADLEY T. SCHEER. Natural heteroagglutinins in the serum of the spiny lobster, Panulirus interruptus. II. Chemical and antigenic relation to blood proteins, 193.

WHITING, ANNA. Dominant lethality and correlated chromosome effects in Habrobracon eggs x-rayed in diplotene and in late metaphase I, 61.

X-RAYS and dominant lethality, 61.

Volume 89 Number 1

THE

BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

E. G. CONKLIN, Princeton University
E. N. HARVEY, Princeton University
SELIG HECHT, Columbia University
LEIGH HOADLEY, Harvard University
L. IRVING, Swarthmore College
M. H. JACOBS, University of Pennsylvania
H. S. JENNINGS, Johns Hopkins University
FRANK R. LILLIE, University of Chicago
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
T. H. MORGAN, California Institute of Technology
G. H. PARKER, Harvard University
A. C. REDFIELD, Harvard University
F. SCHRADER, Columbia University
DOUGLAS WHITAKER, Stanford University

H. B. STEINBACH, Washington University
Managing Editor

AUGUST, 1945

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

SERIAL LIST

A SERIAL list of the holdings of The Marine Biological Laboratory has been published as a separately bound supplement to The Biological Bulletin. This supplement lists with cross references the titles of journals in the Library; additional titles and changes are published annually. A few extra copies of the original list are still available. Orders may be directed to The Marine Biological Laboratory.

MICROFILM SERVICE

THE Library of The Marine Biological Laboratory is now prepared to supply microfilms of material from periodicals included in its extensive list. Through the generosity of Dr. Athertone Seidell, the essential equipment has been set up and put into operation. The Staff of The Marine Biological Laboratory Library is anxious to extend the Microfilm Service, particularly at this time when distance makes the Library somewhat inaccessible to many who normally use it. Investigators who wish films should send to the Librarian the name of the author of the paper, its title, and the name of the periodical in which it is printed, together with the volume and year of publication. The rates are as follows: $.30 for papers up to 25 pages, and $.10 for each additional 10 pages or fraction thereof. It is hoped that many investigators will avail themselves of this service.

Your Biological News

You would not go to the library to read the daily newspaper—probably you have it delivered at your home to be read at your leisure. Why, then, depend upon your library for your biological news?

Biological Abstracts is news nowadays. Abridgments of all the important biological literature are published promptly—in many cases before the original articles are available in this country. Only by having your own copy of *Biological Abstracts* to read regularly can you be sure that you are missing none of the literature of particular interest to you. An abstract of one article alone, which otherwise you would not have seen, might far more than compensate you for the subscription price.

Biological Abstracts is now published in seven low priced sections, as well as the complete edition, so that the biological literature may be available to all individual biologists. Write for full information and ask for a copy of the section covering your field.

BIOLOGICAL ABSTRACTS
University of Pennsylvania
Philadelphia, Pa.

LANCASTER PRESS, Inc.

LANCASTER, PA.

☆

THE EXPERIENCE we have gained from printing some sixty educational publications has fitted us to meet the standards of customers who demand the best.

We shall be happy to have workers at the MARINE BIOLOGICAL LABORATORY write for estimates on journals or monographs. Our prices are moderate.

INSTRUCTIONS TO AUTHORS

The Biological Bulletin accepts papers on a variety of subjects of biological interest. In general, a paper will appear within three months of the date of its acceptance. The Editorial Board requests that manuscripts conform to the requirements set below.

Manuscripts. Manuscripts should be typed in double or triple spacing on one side of paper, 8½ by 11 inches.

Tables should be typewritten on separate sheets and placed in correct sequence in the text. Explanations of figures should be typed on a separate sheet and placed at the end of the text. Footnotes, numbered consecutively, may be placed on a separate sheet at the end of the paper.

A condensed title or running page head of not more than thirty-five letters should be included.

Figures. The dimensions of the printed page, 5 by 7⅜ inches, should be kept in mind in preparing figures for publication. Illustrations should be large enough so that all details will be clear after appropriate reduction. Explanatory matter should be included in legends as far as possible, not lettered on the illustrations. Figures should be prepared for reproduction as line cuts or halftones; other methods will be used only at the author's expense. Figures to be reproduced as line cuts should be drawn in black ink on white paper or blue-lined co-ordinate paper; those to be reproduced as halftones should be mounted on Bristol board and any designating letters or numbers should be made directly on the figures. The author's name should appear on the reverse side of all figures. The desired reduction should be specified on each figure.

Literature cited. The list of literature cited should conform to the style set in this issue of The Biological Bulletin. Papers referred to in the manuscript should be listed on separate pages headed "Literature Cited."

Mailing. Manuscripts should be packed flat. Large illustrations may be rolled in a mailing tube, but all illustrations larger than 9 by 12 inches must be accompanied by photographic reproductions or tracings that may be folded to page size.

Reprints. Authors will be furnished, free of charge, one hundred reprints without covers. Additional copies may be obtained at cost; approximate figures will be furnished upon request.

THE BIOLOGICAL BULLETIN

THE BIOLOGICAL BULLETIN is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to The Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $1.75. Subscription per volume (three issues), $4.50.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Massachusetts, between July 1 and October 1, and to the Department of Zoology, Washington University, St. Louis, Missouri, during the remainder of the year.

Entered as second-class matter May 17, 1930, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

BIOLOGY MATERIALS

The Supply Department of the Marine Biological Laboratory has a complete stock of excellent plain preserved and injected materials, and would be pleased to quote prices on school needs.

* * *

PRESERVED SPECIMENS

for

Zoology, Botany, Embryology,
and Comparative Anatomy

LIVING SPECIMENS

for

Zoology and Botany

including Protozoan and Drosophila Cultures, and Animals for Experimental and Laboratory Use.

MICROSCOPE SLIDES

for

Zoology, Botany, Embryology, Histology, Bacteriology, and Parasitology.

CATALOGUES SENT ON REQUEST

* * *

Supply Department
MARINE
BIOLOGICAL LABORATORY
Woods Hole, Massachusetts

CONTENTS

Page

ANNUAL REPORT OF THE MARINE BIOLOGICAL LABORATORY .. 1

WHITING, ANNA
Dominant lethality and correlated chromosome effects in Habrobracon eggs x-rayed in diplotene and in late metaphase I 61

HARVEY, ETHEL BROWNE
Stratification and breaking of the Arbacia punctulata egg when centrifuged in single salt solutions 72

PACE, D. M.
The effect of cyanide on respiration in Paramecium caudatum and Paramecium aurelia 76

METZ, CHARLES B.
The agglutination of starfish sperm by fertilizin 84

KOZLOFF, EUGENE N.
Cochliophilus depressus gen. nov., sp. nov. and Cochliophilus minor sp. nov., holotrichous ciliates from the mantle cavity of Phytia setifer (Cooper) 95

SCHEER, BRADLEY T.
The development of marine fouling communities 103

SPIEGELMAN, S., AND FLORENCE MOOG
A comparison of the effects of cyanide and azide on the development of frogs' eggs 122

Volume 89 Number 2

THE BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

E. G. Conklin, Princeton University
E. N. Harvey, Princeton University
Selig Hecht, Columbia University
Leigh Hoadley, Harvard University
L. Irving, Swarthmore College
M. H. Jacobs, University of Pennsylvania
H. S. Jennings, Johns Hopkins University
Frank R. Lillie, University of Chicago
Carl R. Moore, University of Chicago
George T. Moore, Missouri Botanical Garden
T. H. Morgan, California Institute of Technology
G. H. Parker, Harvard University
A. C. Redfield, Harvard University
F. Schrader, Columbia University
Douglas Whitaker, Stanford University

H. B. STEINBACH, Washington University
Managing Editor

LIBRARY
WOODS HOLE, MASS.

OCTOBER, 1945

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

SERIAL LIST

A SERIAL list of the holdings of The Marine Biological Laboratory has been published as a separately bound supplement to The Biological Bulletin. This supplement lists with cross references the titles of journals in the Library; additional titles and changes are published annually. A few extra copies of the original list are still available. Orders may be directed to The Marine Biological Laboratory.

MICROFILM SERVICE

THE Library of The Marine Biological Laboratory is now prepared to supply microfilms of material from periodicals included in its extensive list. Through the generosity of Dr. Athertone Seidell, the essential equipment has been set up and put into operation. The Staff of The Marine Biological Laboratory Library is anxious to extend the Microfilm Service, particularly at this time when distance makes the Library somewhat inaccessible to many who normally use it. Investigators who wish films should send to the Librarian the name of the author of the paper, its title, and the name of the periodical in which it is printed, together with the volume and year of publication. The rates are as follows: $.30 for papers up to 25 pages, and $.10 for each additional 10 pages or fraction thereof. It is hoped that many investigators will avail themselves of this service.

Your Biological News

You would not go to the library to read the daily newspaper—probably you have it delivered at your home to be read at your leisure. Why, then, depend upon your library for your biological news?

Biological Abstracts is news nowadays. Abridgments of all the important biological literature are published promptly—in many cases before the original articles are available in this country. Only by having your own copy of *Biological Abstracts* to read regularly can you be sure that you are missing none of the literature of particular interest to you. An abstract of one article alone, which otherwise you would not have seen, might far more than compensate you for the subscription price.

Biological Abstracts is now published in seven low priced sections, as well as the complete edition, so that the biological literature may be available to all individual biologists. Write for full information and ask for a copy of the section covering your field.

BIOLOGICAL ABSTRACTS
University of Pennsylvania
Philadelphia, Pa.

LANCASTER PRESS, Inc.

LANCASTER, PA.

* * *

THE EXPERIENCE we have gained from printing some sixty educational publications has fitted us to meet the standards of customers who demand the best.

We shall be happy to have workers at the MARINE BIOLOGICAL LABORATORY write for estimates on journals or monographs. Our prices are moderate.

INSTRUCTIONS TO AUTHORS

The Biological Bulletin accepts papers on a variety of subjects of biological interest. In general, a paper will appear within three months of the date of its acceptance. **The Editorial Board requests that manuscripts conform to the requirements set below.**

Manuscripts. Manuscripts should be typed in double or triple spacing on one side of paper, 8½ by 11 inches.

Tables should be typewritten on separate sheets and placed in correct sequence in the text. Explanations of figures should be typed on a separate sheet and placed at the end of the text. Footnotes, numbered consecutively, may be placed on a separate sheet at the end of the paper.

A condensed title or running page head of not more than thirty-five letters should be included.

Figures. The dimensions of the printed page, 5 by 7⅜ inches, should be kept in mind in preparing figures for publication. Illustrations should be large enough so that all details will be clear after appropriate reduction. **Explanatory matter should be included in legends as far as possible, not lettered on the illustrations. Figures should be prepared for reproduction as line cuts or halftones; other methods will be used only at the author's expense.** Figures to be reproduced as line cuts should be drawn in black ink on white paper or blue-lined co-ordinate paper; those to be reproduced as halftones should be mounted on Bristol board and any designating letters or numbers should be made directly on the figures. The author's name should appear on the reverse side of all figures. The desired reduction should be specified on each figure.

Literature cited. **The list of literature cited should conform to the style set in this issue of The Biological Bulletin. Papers referred to in the manuscript should be listed on separate pages headed "Literature Cited."**

Mailing. Manuscripts should be packed flat. Large illustrations may be rolled in a mailing tube, but all illustrations larger than 9 by 12 inches must be accompanied by photographic reproductions or tracings that may be folded to page size.

Reprints. Authors will be furnished, free of charge, one hundred reprints without covers. Additional copies may be obtained at cost; approximate figures will be furnished upon request.

THE BIOLOGICAL BULLETIN

THE BIOLOGICAL BULLETIN is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to The Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $1.75. Subscription per volume (three issues), $4.50.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Massachusetts, between July 1 and October 1, and to the Department of Zoology, Washington University, St. Louis, Missouri, during the remainder of the year.

Entered as second-class matter May 17, 1930, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

BIOLOGY MATERIALS

The Supply Department of the Marine Biological Laboratory has a complete stock of excellent plain preserved and injected materials, and would be pleased to quote prices on school needs.

PRESERVED SPECIMENS

for

Zoology, Botany, Embryology,
and Comparative Anatomy

LIVING SPECIMENS

for

Zoology and Botany

including Protozoan and Drosophila Cultures, and Animals for Experimental and Laboratory Use.

MICROSCOPE SLIDES

for

Zoology, Botany, Embryology, Histology, Bacteriology, and Parasitology.

CATALOGUES SENT ON REQUEST

Supply Department
MARINE
BIOLOGICAL LABORATORY
Woods Hole, Massachusetts

CONTENTS

Page

KIDDER, GEORGE W., AND VIRGINIA C. DEWEY
Studies on the biochemistry of Tetrahymena. IV. Amino acids and their relation to the biosynthesis of thiamine..... 131

LEFEVRE, PAUL G.
Certain chemical factors influencing artificial activation of Nereis eggs.................................... 144

HIBBARD, HOPE AND GEORGE I. LAVIN
A study of the Golgi apparatus in chicken gizzard epithelium by means of the quartz microscope...................... 157

HAYASHI, TERU
Dilution medium and survival of the spermatozoa of Arbacia punctulata. I. Effect of the medium on fertilizing power.. 162

KOZLOFF, EUGENE N.
Heterocineta phoronopsidis sp. nov., a ciliate from the tentacles of Phoronopsis viridis Hilton...................... 180

ABSTRACTS OF SCIENTIFIC PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY, SUMMER OF 1945........... 184

Volume 89 Number 3

end

THE BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

E. G. CONKLIN, Princeton University
E. N. HARVEY, Princeton University
SELIG HECHT, Columbia University
LEIGH HOADLEY, Harvard University
L. IRVING, Swarthmore College
M. H. JACOBS, University of Pennsylvania
H. S. JENNINGS, Johns Hopkins University
FRANK R. LILLIE, University of Chicago
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
T. H. MORGAN, California Institute of Technology
G. H. PARKER, Harvard University
A. C. REDFIELD, Harvard University
F. SCHRADER, Columbia University
DOUGLAS WHITAKER, Stanford University

H. B. STEINBACH, Washington University
Managing Editor

Marine Biological Laborato
LIBRARY
JAN 15 1946
WOODS HOLE, MASS.

DECEMBER, 1945

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

SERIAL LIST

A SERIAL list of the holdings of The Marine Biological Laboratory has been published as a separately bound supplement to The Biological Bulletin. This supplement lists with cross references the titles of journals in the Library; additional titles and changes are published annually. A few extra copies of the original list are still available. Orders may be directed to The Marine Biological Laboratory.

MICROFILM SERVICE

THE Library of The Marine Biological Laboratory is now prepared to supply microfilms of material from periodicals included in its extensive list. Through the generosity of Dr. Athertone Seidell, the essential equipment has been set up and put into operation. The Staff of The Marine Biological Laboratory Library is anxious to extend the Microfilm Service, particularly at this time when distance makes the Library somewhat inaccessible to many who normally use it. Investigators who wish films should send to the Librarian the name of the author of the paper, its title, and the name of the periodical in which it is printed, together with the volume and year of publication. The rates are as follows: $.30 for papers up to 25 pages, and $.10 for each additional 10 pages or fraction thereof. It is hoped that many investigators will avail themselves of this service.

Your Biological News

You would not go to the library to read the daily newspaper—probably you have it delivered at your home to be read at your leisure. Why, then, depend upon your library for your biological news?

Biological Abstracts is news nowadays. Abridgments of all the important biological literature are published promptly—in many cases before the original articles are available in this country. Only by having your own copy of *Biological Abstracts* to read regularly can you be sure that you are missing none of the literature of particular interest to you. An abstract of one article alone, which otherwise you would not have seen, might far more than compensate you for the subscription price.

Biological Abstracts is now published in seven low priced sections, as well as the complete edition, so that the biological literature may be available to all individual biologists. Write for full information and ask for a copy of the section covering your field.

BIOLOGICAL ABSTRACTS
University of Pennsylvania
Philadelphia, Pa.

LANCASTER PRESS, Inc.

LANCASTER, PA.

☆

THE EXPERIENCE we have gained from printing some sixty educational publications has fitted us to meet the standards of customers who demand the best.

We shall be happy to have workers at the MARINE BIOLOGICAL LABORATORY write for estimates on journals or monographs. Our prices are moderate.

INSTRUCTIONS TO AUTHORS

The Biological Bulletin accepts papers on a variety of subjects of biological interest. In general, a paper will appear within three months of the date of its acceptance. **The Editorial Board requests that manuscripts conform to the requirements set below.**

Manuscripts. Manuscripts should be typed in double or triple spacing on one side of paper, 8½ by 11 inches.

Tables should be typewritten on separate sheets and placed in correct sequence in the text. Explanations of figures should be typed on a separate sheet and placed at the end of the text. Footnotes, numbered consecutively, may be placed on a separate sheet at the end of the paper.

A condensed title or running page head of not more than thirty-five letters should be included.

Figures. The dimensions of the printed page, 5 by 7⅜ inches, should be kept in mind in preparing figures for publication. Illustrations should be large enough so that all details will be clear after appropriate reduction. **Explanatory matter should be included in legends as far as possible, not lettered on the illustrations. Figures should be prepared for reproduction as line cuts or halftones; other methods will be used only at the author's expense.** Figures to be reproduced as line cuts should be drawn in black ink on white paper or blue-lined co-ordinate paper; those to be reproduced as halftones should be mounted on Bristol board and any designating letters or numbers should be made directly on the figures. The author's name should appear on the reverse side of all figures. The desired reduction should be specified on each figure.

Literature cited. **The list of literature cited should conform to the style set in this issue of The Biological Bulletin. Papers referred to in the manuscript should be listed on separate pages headed "Literature Cited."**

Mailing. Manuscripts should be packed flat. Large illustrations may be rolled in a mailing tube, but all illustrations larger than 9 by 12 inches must be accompanied by photographic reproductions or tracings that may be folded to page size.

Reprints. Authors will be furnished, free of charge, one hundred reprints without covers. Additional copies may be obtained at cost; approximate figures will be furnished upon request.

THE BIOLOGICAL BULLETIN

THE BIOLOGICAL BULLETIN is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to The Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $1.75. Subscription per volume (three issues), $4.50.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Massachusetts, between July 1 and October 1, and to the Department of Zoology, Washington University, St. Louis, Missouri, during the remainder of the year.

Entered as second-class matter May 17, 1930, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

BIOLOGY MATERIALS

The Supply Department of the Marine Biological Laboratory has a complete stock of excellent plain preserved and injected materials, and would be pleased to quote prices on school needs.

* * *

PRESERVED SPECIMENS

for

Zoology, Botany, Embryology,
and Comparative Anatomy

LIVING SPECIMENS

for

Zoology and Botany

including Protozoan and Drosophila Cultures, and Animals for Experimental and Laboratory Use.

MICROSCOPE SLIDES

for

Zoology, Botany, Embryology, Histology, Bacteriology, and Parasitology.

CATALOGUES SENT ON REQUEST

* * *

Supply Department
MARINE
BIOLOGICAL LABORATORY
Woods Hole, Massachusetts

CONTENTS

Page

TYLER, ALBERT AND BRADLEY T. SCHEER
Natural heteroagglutinins in the serum of the spiny lobster, Panulirus interruptus. II. Chemical and antigenic relation to blood proteins 193

ROGICK, MARY DORA
Studies on marine Bryozoa. I. Aeverrillia setigera (Hincks) 1887 201

ROGICK, MARY DORA
Studies on fresh-water Bryozoa. XVI. Fredericella australiensis var. browni, n. var. 215

KIDDER, GEORGE W. AND VIRGINIA C. DEWEY
Studies on the biochemistry of Tetrahymena. VII. Riboflavin, pantothen, biotin, niacin and pyridoxine in the growth of T. geleii W. 229

RIS, HANS
The structure of meiotic chromosomes in the grasshopper and its bearing on the nature of "chromomeres" and "lampbrush chromosomes" 242

MBL/WHOI LIBRARY
WH 17J7 /